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UNIVERSITY OF CINCINNATI

May 21 1946

I hereby recommend that the thesis prepared under my supervision by Henry S. Bloch

entitled Studies on chemical methods for the Diagnosis and Treatment of Carcinoma of the Stomach

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

Approved by:

Francis E. Ray

Hoke S. Greene

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STUDIES ON CHEMICAL METHODS FOR THE DIAGNOSIS AND TREATMENT OF CARCINOMA OF THE STOMACH

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

1946

by

Henry S. Bloch

Dipl. Chem. Technikum of the Kanton Zurich, 1939

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The studies on the gastric excretion of sulfonamides were carried out in close cooperation with Dr. Nathan Shapiro of the Cincinnati General Hospital. Much of the author's medical knowledge was gained from this association with Dr. Shapiro.

The radioiodide was supplied by the Department of Physics of the Massachusetts Institute of Technology through the courtesy of Professor Robley D. Evans. Dr. W. Peacock of the same Institute was kind enough to check some of the early radioactivity measurements.

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CONTENTS

1) ONCOLOGY

1.1) HISTORICAL DEVELOPMENT

Oncology is the science dealing with pathologic tumors. An autonomous new growth is called a tumor or neoplasm. Depending on the extent to which neoplasms influence the normal physiology of the host they are called benign or malignant. The difference is merely one of degree and there is a gradual transition from benign to malignant types of neoplasia.

Neoplastic diseases are mentioned in the oldest remnants of Indian and Persian literature, and in the Papyrus Ebers (1500 B.C.).(2) 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 use the term καρκίνος for indolent ulcers and καρκίνωμα for progressive malignant tumors. Hippocrates' terminology has survived in the modern concept of cancer. The word carcinoma is today restricted to tumors of glandular epithelium in a typical arrangement.

The riddle of neoplasia occupied the minds of physicians and surgeons through the antique and middle ages. Methods of treatment were subject to the medical fashions of the times. However, some surgical procedures appear to have

been remarkably successful.

The advent of printed books during the Renaissance greatly stimulated oncology. Astruc (1684-1766) 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 at autopsies and conceived the role of the lymph in the formation of metastases. An essay by Peyrilhe, written in the 18th century and submitted to the Academy of Lyons in answer to the question "Qu' est ce que le cancer?" constitutes the first systematic treatment of the subject. By animal experimentation Peyrilhe tried to demonstrate a virus principle originating from tumors. He also treated ulcerating lesions successfully with carbolic acid. John Hunter in England (1728-1793) put forth the belief that tumor tissues were nouriched by the body like normal tissues and develop following the same biological laws. Pott in 1775 correctly described the cancer of chimney sweeps and traced its etiology, an observation which, some 150 years later, led to the work on carcinogenic hydrocarbons.

The "Society for Investigating the Nature and Cause of Cancer" existed in London from 1802 to 1806. "It formulated the problems of the disease as they stand today." (Ewing 1940 l.c.).

In 1820 Mannoir announced that each tumor results through morbid changes of the tissues from which it arises.

He tried to prove his point by crude chemical and physical tests. Microscopic studies by Raspail showed 6 years later that growth of tissues results from the multiplication of cells. In 1838 J. Mueller published his classical studies on microscopic findings in malignant tumors. During the period of Virchow careful histologic studies were further extended. At the time it was believed that cancer originated from a fluid blastema in the blood. Chemists were called upon to separate from the blood the different blastemas which were held responsible for different tumors. Fuehrer claimed that he could distinguish three varieties of blastemas by means of color reactions with nitric acid.

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, and the separate position of sarcomas (tumors of connective tissues) and epitheliomas (tumors of epithelial cells) were accepted facts. Further progress in the concept of histogenesis had to wait until Virchow announced his principle "Omnis cellula e cellula" and therby founded cellular pathology. Through improved histologic techniques Thiersch could demonstrate the invariable derivation of epitheliomas from lining epithelium and thus extended Virchow's principle to "omnis cellula e cellula ejusdem generis". Thiersch held that the equilibrium between stroma dnd epithelium became disturbed by a process of involution, resulting in overgrowth

of epithelium. He thus enunciated the first competent physiological theory of the pathogenesis of cancer.

Cohnheims doctrine (1877) that tumors do not arise from normal cells but mainly from isolated and usually embryonical cells further clarified the ideas about malignant growth and separated it from all other pathological processes. While Cohnheim's theory is not applicable to all tumors it forms one of the most substantial chapters of present day knowledge concerning the nature and origin of true neoplasia.

By 1900 the natural history of malignant neoplasms had been written and tumors had been classified on a histogenic basis. It had been shown that neoplasia is of local origin and that metastases arise from transported cells. It was recognized that irritation, trauma, and infection seemed to be connected with the disease, but the relation of these factors remained obscure.

With the beginning of the 20th century experimental biology and biochemistry entered actively into cancer research. The most recent advanced have come from these disciplines.

1.2) RECENT ADVANCED IN ONCOLOGY

The efforts of biochemistry and experimental biology in cancer research may be divided into three categories: Studies on the pathogenesis of neoplasia, on the diagnosis of the disease, and on methods of treatment.

1.21) Investigations on the Pathogenesis of Tumors.

Some aspects of the pathogenesis of tumors have been

investigated with remarkable success. During the last decade of the 19th century the numerous attempts which had been made to transplant tumors from humans to animals and among animals finally gave positive results when Morau carried a mouse carcinoma through several generations.(3)

The work was further extended by Loeb, Jensen, and others, and transplanting of tumors is an established tool in modern cancer research.

The carcinogenic effects of radiation were encountered even prior to the discovery of either x-rays of radium, both of which are now recognized as potent carcinogens. Workers of the Schneeberg mines in Germany for years had been known to have a strangely prevalent fatal disease of the lung. It was later identified as cancer of the lung caused by inhalation of radioactive particles from the ore of the mines.(4)

Shortly before World War One Fibiger found that feeding of cockroaches which were infested with certain parasites to rats resulted in the production of carcinoma of the stomach. Fibiger designated the parasite as spiroptera neoplastica.*(5)

At the time Fibiger published his studies, Yamagiwa and Ichikawa announced the production of carcinomas in rabbits by application of coal tar.(7) Some 15 years later Kennaway

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*In my experiments with mice I have observed several with "spontaneous" but otherwise undesignated tumors. On autopsy I found many of these animals to have a remarkably large spleen and stomach. The stomach was often filled with worms, up to 4 cm long and about 2 mm in diameter. I have had no opportunity to follow up these observations.

and Cook[8] discovered the carcinogenicity of certain polycyclic hydrocarbons. In this group methylcholanthrene was by and large found to be the most potent compound. The mechanism by which the carcinogenic hydrocarbons exert their biologic action is not yet understood. Fieser points out that they are rather active substances, resembling phenols and amines in their chemical properties. This may offer a clue to their mode of action.[9]

Some very interesting studies on the production of malignant changes in normal cells in vitro were recently made by Earle.[10] With his coworkers he grew normal cells of C3H strain mice in vitro on a heterologous medium containing methylcholanthrene in small concentrations. The cells grew in an abnormal fashion. When samples of the cultures were injected into C3H mice, tumors grew at the site of injection, and some of these neoplasms metastasized.

A survey of chemical compounds which had been tested for carcinogenic activity up to 1939 was published by Hartwell in 1941.[11] It lists data on some 700 substances, many of which were found to be active.

Clinicians have pointed out the possibilities of hereditary factors in neoplasia ever since Warren's report on a "cancer family" in 1837.[12] In 1914 Little began to bread mice with the aim of developing cancer resistant and cancer susceptible strains. This work has been highly successful. Several strains of animals with naturally high

and naturally low incidence of various types of malignancies are today available for research.(13)

By the use of pure strain mice Bittner (1936) could show that an extrachromosomal factor plays an important role in rendering mice susceptible to neoplasia. This extrachromosomal 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 foster nursed animals show a low tumor incidence. The complementary procedure, namely feeding of high tumor strain milk to low tumor strain infant mice produces high tumor strain animals. The results obtained by foster nursing experiments were parallelled by studies on reciprocal mating.(14)

1.22) Studies on the Diagnosis of Malignant Diseases.

The numerous attempts to develop diagnostic tests for cancer have been competently reviewed by Stern and Willheim.(15) At present there exists no rational basis for the hope that a diagnostic test for malignant diseases, similar in effect to the Wasserman test for lues, can be developed. However, if a tumor affects a phase of the physiology of the host which can be evaluated with the scientific tools now available the course of the disease can be followed. Thus, the prostate normally produces acid phosphatase. The presence of acid phosphatase in the serum is a secondary male sex characteristic of a chemical nature. In prostatic carcinoma,

and particularly in the presence of metastases, which produce acid phosphatase like the normal gland, the serum acid phosphatase level becomes elevated due to excess formation of the enzyme. Furthermore, prostatic carcinoma tends to metastasize to bone. Closely connected with bone production is the alkaline phosphatase and the level of this enzyme is a rather accurate index for the activity of bone tissue in the organism. The metastatic carcinoma cells of the prostate stimulate the osteoblasts, and thereby raise the serum level of alkaline phosphatase. The acid and alkaline phosphatase levels in patients with carcinoma of the prostate can be used in following the course of the disease and in making a prognosis.(16)

1.23) Advanced in the Treatment of Neoplastic Diseases.

Advances in the treatment of neoplastic diseases have generally parallelled the progress of surgery. With improved operative techniques and better general care for the patient, more tumors have become accessible to the knife; the mortality rate at operation has dropped, and the comfort of the victim of the disease has increased. The discussion of the curability of cancer as a whole has, however, little significance. It gains meaning only when different types of tumors are considered as separate entities. At present percentages of cures range from 80 for lip cancer to practically zero for carcinoma of the esophagus. Sixty-five per cent of all cancer cases fall into the group of internal tumors with

which curative medicine can not cope at present.(17)

The first successful attempt at a chemical control of cancer was precipitated by Huggins in 1940.(18) 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.

Otherwise, radiation is so far the closest approach to a rational curative agent for neoplastic diseases. Therapeutic x-ray apparatus and radium are standard equipment on every tumor service. Both suffer however, from some innate shortcomings. In most cases normal tissues are exposed to rather intense pathogenic radiation when x-ray therapy is used. Skin tolerance is then often reached before sufficient radiation to check tumor growth has been administered. This is particularly true in the treatment of internal neoplasms, such as carcinoma of the stomach. The use of radium has similar limitations. Many tumors can not be reached with radium applicators.

Studies to overcome the deficiencies of x-ray and radium therapy by the use of radioactive isotopes are now under way. This thesis will deal with some aspects of the

work in considerable detail later. But the already established use of radiophosphorus in the treatment of leukemia and lymphosarcoma shall be mentioned here. The malignant cells in these two diseases show a high and rapid uptake of radiophosphorus following its administration as a phosphate. Thus, when radiophosphate is injected into victims of these maladies, the neoplastic tissue is exposed to intense radiation with a rather high degree of selectivity. Unfortunately, bone takes up larger amounts of radiophosphorus than do the tumors. The rather intense radiation to which the bone marrow becomes thereby exposed limits the use of radiophosphate in the treatment of leukemia and allied conditions.(19)

The radiosensitivity of tumors is of prime importance in the radiotherapeutic attack on cancer. But there is no simple scheme which permits one to judge whether or not or to what degree a tumor is radiosensitive. Our knowledge concerning the action of radiation on living cells is still in its infancy. Shields Warren has reviewed the factual information available on the effects of radiation on normal tissues.(20) It is important to remember that the effect of radiation does not depend on the dose given, but on the amount absorbed (Grotthuss - Draper law).(21) It is known that all biological effects of alpha-, beta-, gamma-, and x-rays as well as neutrons are caused essentially by the ionization produced in the tissues. Alpha- and beta-rays produce ionization directly, the other radiations indirectly.(22)

An x-ray photon, on collission with an electron, may transfer all or part of its energy to the electron, depending on the energy of the photon (photoelectric effect and Compton effect respectively.). The secondary electrons thus formed give rise to chemical changes, which in turn produce biological effects. The secondary electrons generated by x-rays are physically similar to the beta-rays emitted by radioactive isotopes. X-ray dosage is usually measured in roentgens, one roentgen being equivalent to 83 ergs per gram of tissue. According to Evans, "1 equivalent roentgen of beta radiation per day is delivered to tissue containing 57/E microcuries per kilogram of a pure beta-ray emitter whose maximum beta-ray energy in mev is E."*(23)

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*A radiation dose of 0.1 roentgen per day is considered roughly to produce no biologic effects. Thus, the maximum safe concentration of an isotope which emitts beta-rays only would be C = 5.7/E microcuries per kg. Pollard and Davidson(24) add a word of caution to the above figures, particularly with regard to possible genetic changes. "The exposure of an individual to radiation automatically renders him liable to suffer a mutation, and there is no threshold to the radiation dose which will produce the change. It is just a gamble. There is thus no safe dose that can be prescribed; all one can do is to consider what has been taken safely in the past, and this is not a negligible amount, for at high altitudes cosmic rays produce about 1/1000 roentgen per day, and this seems to be safe. We therefore suggest that the younger nuclear physicist and radiologist limit his daily dose to something nearer 1/100 roentgen per day. The health of workers exposed to radiation should be checked by blood counts about every month. The number of white cells and the relative proportion of polymophonuclear cells become diminished by radiation, and any such drop should be watched."

While it is thus known that radiobiologic action begins with changes in the atomic structure of matter and terminates with modified behavior of cells, the intermediary steps of the process are unknown. Intelligent discussion of the effects of radiation on normal and pathologic tissues will have to wait until the action of radiation on the various factors of growth are understood in more detail. In the meantime practical rules about radiosensitivity must be learned from the hard school of experience.(25)

1.24) General Views on Neoplastic Diseases.

Despite the progress which has been achieved in some disciplines of oncology, the nature of "spontaneous" neoplastic growth in man remains obscure, indeed so much so that today two schools of thought look on the whole problem from two opposite points of view. The unitarian school holds that cancer is one disease entity with subsidiary variations. The proponents of the virus theory of cancer may be said to belong to this school.(26) On the other hand, a considerable number of modern investigators feel that neoplasia comprises a large group of distinct disease entities. To quote Ewing:(27)

"No one would think of confusing lobar pneumonia with pneumonic plague, although both are examples of acute exudative pneumonitis, but it is quite the rule to identify for statistical study several equally divergent forms of mammary cancer. The former diseases are related only as forms of inflammation, the latter only as types of neoplasia. From this point of view it may safely be said that there are more distinct clinical and pathologic entities within the groups of neoplasms than exist outside of them."

In view of the multitude of biological, chemical, and physical agents which are known to produce cancer, and in view of the fact that it has not been possible so far to reduce neoplastic diseases to a common denominator, Ewing's attitude seems to be the sounder basis for experimental cancer research. Even if Ewing should be mistaken, consideration of different tumors as separate disease entities will not retard cancer research, and may avoid considerable confusion in the interpretation of experimental data. The National Cancer Institute has studied mammary tumors in mice from this point of view in recent years. In this thesis carcinoma of the stomach in humans shall be regarded in a similar fashion.

2) CARCINOMA OF THE STOMACH IN MAN

Depending on the interpretation of statistics, the stomach can be considered the most common site of neoplastic growth in man, and gastric tumors are among the most difficult to control. They have a tendency to metastasize early, involving the omentum and peritoneum, liver, lymph nodes, rectum, ovaries, lungs, bones, adrenals, and brain, roughly in this order.

The more important diseases of the stomach are enumerated in table I. Although they differ widely from the point of view of histology, etiology and prognosis, their early symptoms are very similar, and differential diagnosis at the onset of illness is correspondingly difficult. Advanced carcinoma of the stomach is about the easiest disease to recognize, but in its beginnings it is likely to elude both patient and physician. While x-ray and gastroscopy have generally improved the results in the diagnosis of gastric disorders, neither could as yet be developed into an effective tool for the early discovery of gastric cancer.(28)

Evaluation of the gastric function was a logical step in attempts to improve methods for the diagnosis of diseases of the stomach. During the eighteen seventies Leube began to aspirate gastric contents of patients through a stomach tube in order to study digestive activity. He gave

Table I

Diseases of the Stomach

1) Gastritis

- 1.1) Acute Gastritis
  - 1.11) Acute simple exogenous gastritis (food poisoning)
  - 1.12) Acute corrosive gastritis (acids, lyes)
  - 1.13) Acute infectious gastritis (caused by toxins or bacteria entering the stomach from the blood stream, e.g. in measles, pneumonia, etc.)
- 1.2) Chronic Gastritis (classified chiefly on gastroscopic observation)
  - 1.21) Chronic superficial gastritis
  - 1.22) Chronic atrophic gastritis
  - 1.23) Chronic hypertrophic gastritis

2) Peptic Ulcer

- 2.1) Duodenal Ulcer
- 2.2) Benign Gastric Ulcer

3) Carcinoma of the Stomach (Ewing's classification)

- 3.1) Adenocarcinoma
- 3.2) Gelatinous Carcinoma
- 3.3) Ulcerocancer
- 3.4) Carcinoma simplex. Diffuse Carcinoma.
- 3.5) Diffuse scirrhus carcinoma
- 3.6) Sclerosing fibrocarcinoma. Linitis Plastica.

4) Sarcoma of the Stomach (Ewing's classification)

- 4.1) Myosarcoma
- 4.2) Lymphosarcoma
- 4.3) Miscellaneous sarcomas.

5) Benign Tumors (According to G.B. Eusterman in Portis')

- 5.1) Polyp
- 5.2) Smooth muscle tumors
- 5.3) Adenomas
- 5.4) Polyposis
- 5.5) Hemangioma
- 5.6) Fibroma
- 5.7) Neurofibroma
- 5.8) Dermoid cyst
- 5.9) Papilloma

6) Gastric Lues

his subjects a meal consisting of soup, steak, and a bun. After several hours the gastric contents were removed and examined for digested and undigested food.[29] Ewald and Boas began to titrate the acid in gastric juice with n/10 sodium hydroxide and introduced the test meal which still carries their name today.[30] Since then a large number of publications dealing with the acidity of gastric juice in health and disease have appeared. The results have been summarized by Bloomfield: "Analysis of the gastric secretions gives usually little or no information of clinical use. In certain special situations - for instance, when duodenal ulcer or pernicious anemia is suspected - determination of gastric acidity may be helpful."[31]

In 1908 a short note on the excretion of Neutral Red in the Pavlov pouch of a dog was published by Fuld. The gastric excretion of this compound in experimental animals and man has since been discussed in some 50 papers.[32] During the nine-teen-twenties and - thirties dyes in general were employed extensively in the study of gastric physiology.[33] By 1940 it was generally recognized that "basic" dyes tend to be excreted by the gastric mucosa, but that "acid" dyes as a rule do not enter into the gastric juice from the blood. No significant advances concerning the mechanism of gastric secretion or the use of dyes as diagnostic tools had otherwise been made.

3) THE EXCRETION OF SULFONAMIDES BY THE HUMAN STOMACH

3.1) INTRODUCTION

Except for the fact that they do not absorb visible light, sulfonamides are very much like dyes from a physico-chemical point of view. The secretion of some of these drugs by the dog's stomach was studied by Carrier and Ivy, and by Cooke, Davenport and Goodman.[34] Since sulfonamides are amphoteric compounds of medium molecular weight, Dr. Shapiro, Dr. Schiff and myself felt that they held promise as possible tools for the study of humas gastric function. Furthermore, the pharmacologic properties of these compounds were well known, they could be obtained in a form suitable for injection into patients, and they could readily be analyzed for in biological fluids. Early in 1942 we therefore began to study the gastric excretion of sulfonamides in man. The experimental details of this work have been published in Gastroenterology.[35]

3.2) RESULTS

3.21) SULFATHIAZOLE

Nine studies in 6 subjects, comprising 3 normals, 2 patients with atrophic gastritis, and 1 with an advanced carcinoma of the stomach, showed that only small amounts of

sulfathiazole enter into the gastric secretion. In the individual experiments maximum concentrations of the drug in gastric juice ranged from 0.7 to 1.7 mg %; the concentration ratios $\frac{\text{mg\% sulfonamide in gastric juice}}{\text{mg\% sulfonamide in blood}}$ ranged from 0.1 to 0.2. Stimulation of the parietal cells with histamine did not seem to influence the sulfathiazole level in the gastric contents. The amount of sulfathiazole excreted was so small that it could not be used to evaluate gastric function. Because of possible complications it was felt that the dosage of sulfathiazole (2 gm) should not be raised, and work on this compound was abandoned.

3.22) SULFADIAZINE

A group of 43 subjects was studied, comprising 7 normals (2 with achlorhydria), 10 patients with duodenal ulcer, 5 with gastric ulcer, 8 with atrophic gastritis (6 with achlorhydria), and 13 with gastric cancer (9 with achlorhydria).

Table II shows the concentrations of sulfadiazine in the gastric juice of the individual subjects at various time intervals after injection. The peak concentration of the sulfonamide in the gastric juice was usually reached within 15 to 45 minutes. It ranged from 4 to 21 mg%, the highest values occuring in patients with gastric carcinoma.

Table III gives the distribution of the concentration ratios in all of the subjects studied. The highest values occurred in patients with gastric carcinoma with

achlorhydria. In the 4 cases of gastric carcinoma with free hydrochloric acid in the gastric contents the ratios were no higher than those obtained in the subjects without carcinoma.

In 10 subjects studies were made on different days to obtain some idea as to the possible variation of the concentration ratio in a given individual. The concentration ratios obtained on different days in the same subjects are listed in table IV. Almost identical values were obtained in 6 subjects. The maximum difference was 0.24.

The mean concentration ratios in the various groups are shown in table V. The normals and the patients with peptic ulcer, atrophic gastritis, or gastric carcinoma with free hydrochloric acid had a mean concentration ratio of 0.45 or less. In 7 cases of gastric carcinoma and achlorhydria in which the lesion was not confined to the pylorus the mean concentration ratio was 0.69; in 2 similar cases in which the tumor was confined to the pyloric region, the mean concentration ratio was 0.27.

Table VI is a diagramatic representation of the extent of the cancers in the various stomachs. With three exceptions (designated by asterisks) estimation of the size of the lesion was based on findings at operation or autopsy. In the three exceptions, the extent of the tumor was estimated on the basis of x-ray and gastroscopic findings.

In a few instances (not included in the tables) 0.5 mg histamine was injected subcutaneously at the end of

Table II

Concentration of sulfadiazine in the gastric juice (individual determinations)

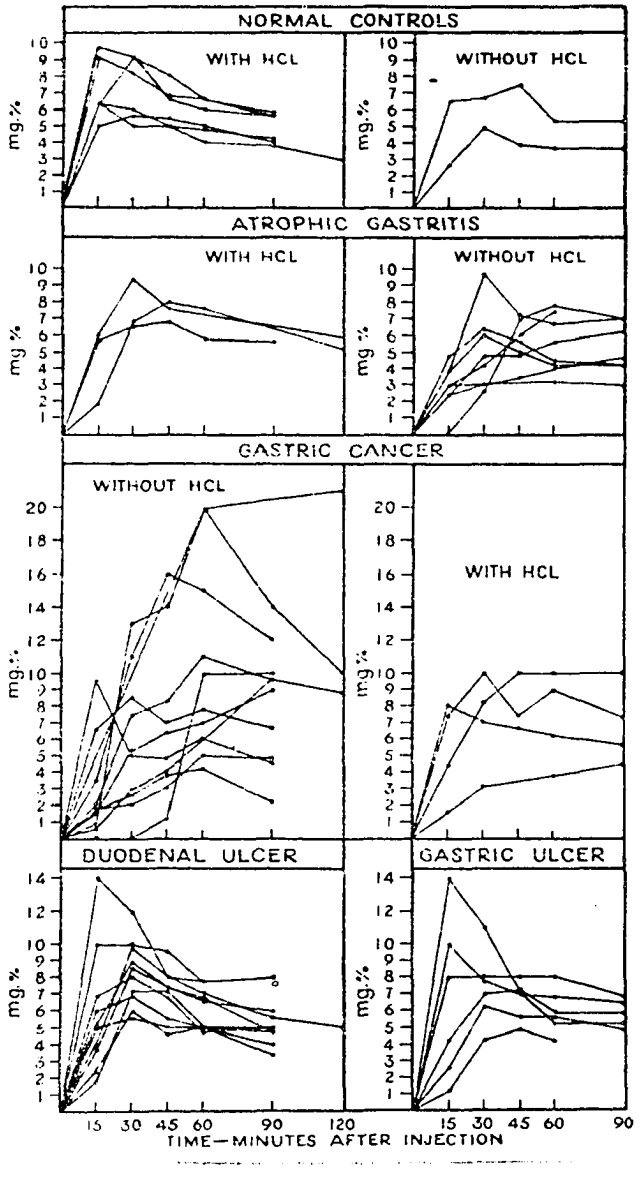


Table III

Distribution of concentration ratios of sulfadiazine in normals and patients with peptic ulcer, atrophic gastritis, and gastric cancer

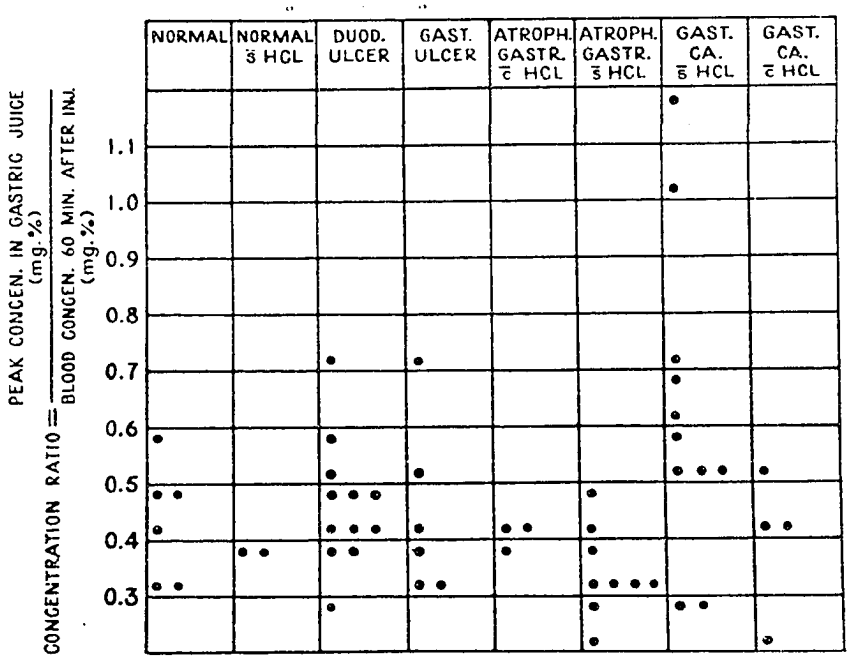


Table IV

Concentration ratios obtained on different days on the same subjects (as listed by their initials)

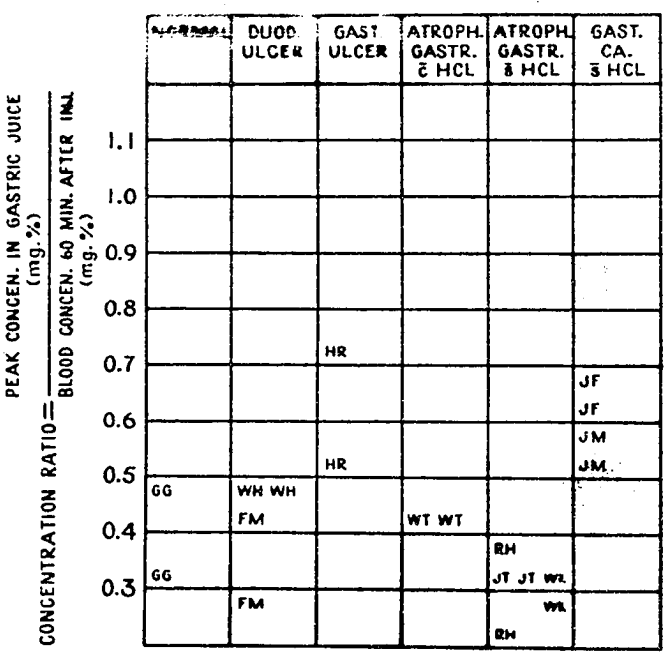


Table V

Mean concentration ratios in the various groups of patients

	Number of Patients	Number of Determinations	Mean Concentration Ratios
Normal controls	5	6	0.43
Normal stomach (gastroscopically) with achlorhydria	2	2	0.38
Duodenal ulcer	10	12	0.45
Gastric ulcer	5	6	0.45
Atrophic gastritis with free HCl	2	3	0.40
Atrophic gastritis with achlorhydria	6	9	0.34
Gastric cancer with achlorhydria	7	9	0.69
Gastric cancer with achlorhydria (lesion confined to pyloric region)	2	2	0.27
Gastric cancer with free HCl	4	4	0.39

the sulfadiazine injection and was found to have no significant effect on the concentration of the sulfonamide in the gastric juice.

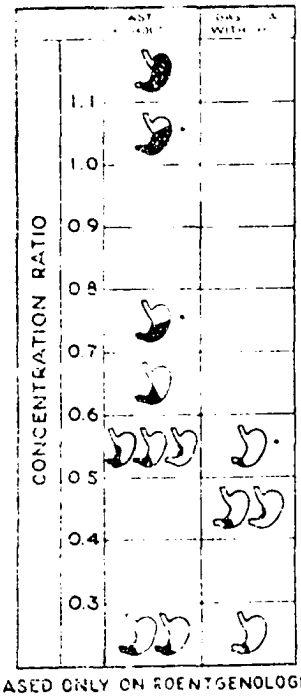
3.23) SULFAPYRIDINE

The gastric excretion of this compound was observed in a group of 37 subjects, comprising 8 normals, 10 patients with duodenal ulcer, 5 with gastric ulcer, 1 with both gastric and duodenal ulcer, 5 with atrophic gastritis, and 8 with gastric cancer. The peak concentration of sulfapyridine in the gastric juice occurred about 1 hr. following the injection of the drug. The concentration ratio of sulfapyridine was usually much greater than 1 and varied with the acidity of the gastric juice (table VII). It seemed to be largely independent of the anatomical state of the stomach. In the presence of free hydrochloric acid (pH 1 to 3) the concentration ratios were usually between 4 and 8. As the pH rose above 3, the concentration ratios gradually fell until they reached values around unity at a pH of 7 to 8.

Five patients were given 0.5 mg histamine subcutaneously at the time of injection of the sulfapyridine. When the histamine lowered the pH of the gastric juice without too great an increase in the rate of secretion, a definite rise in the concentration ratios occurred. Similar changes in the concentration ratios were seen in 7 patients who received 0.5 mg histamine subcutaneously 1 1/2 hr. after the

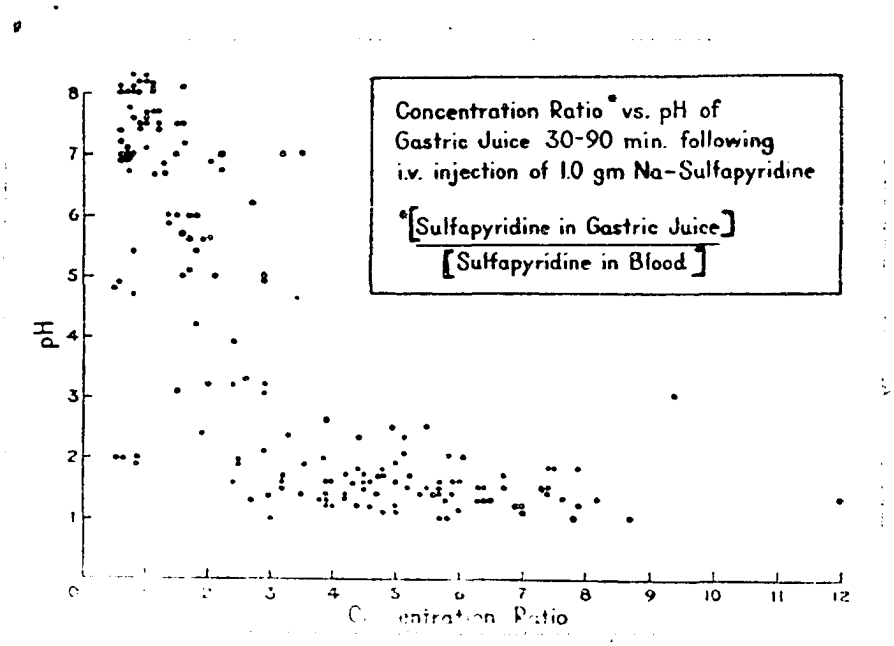
Table VI

Correlation between the Concentration Ratio of Sulfadiazine and the Extent of the Gastric Cancers



*BASED ONLY ON ROENTGENOLOGICAL AND GASTROSCOPIC FINDINGS

Table VII



injection of sulfapyridine, i.e. after the spontaneous excretion of sulfapyridine alone had been observed.

3.3) DISCUSSION

The data reported above make it obvious that none of the three sulfonamides as studied are suitable for the early diagnosis of gastric cancer. While the concentration ratio of sulfadiazine was definitely elevated in several patients with gastric malignancies, the tumors in these cases were quite extensive and did not present a diagnostic problem. The excretion of sulfapyridine was largely determined by the secretion of hydrochloric acid. Further investigation of the gastric excretion of this drug may show that it can be developed into a highly sensitive test for the function of the parietal cells, and such a test may prove superior to the methods now in use such as the Ewald test and the histamine test.

The results obtained with sulfapyridine led me to suspect that the gastric excretion of this drug is essentially a function of its physico-chemical properties and of the pH of the gastric contents. In 1942 Davenport published a theory concerning the gastric secretion of sulfonamides "based on the activities of the drugs in plasma and gastric juice and upon their rates of diffusion through the tissues."(36) This theory explained all the observations which Cooke, Davenport and Goodman (l.c.) had made on dogs. While the results of

these authors are generally in good agreement with the data reported above for humans, Cooke and his coworkers did not report a change of the concentration ratio with changing pH of the gastric secretion. This is not surprizing because their studies were performed on normal pouch dogs which are known to have a gastric secretion with a consistently low pH following the injection of histamine.

Since the sulfonamides are amphoteric compounds it seemed to me that it should be possible to develop an equation for the change of the concentration ratio with the pH of the gastric contents. These deliberations were based on Nernst's concept of the distribution law for a dissociable compound, such as governs for instance the distribution of benzoic acid between water and benzene.[37] It can be shown that in such a case there exists a relationship

$$\frac{(1 - \alpha_I)\, C_I}{(1 - \alpha_{II})\, C_{II}} = \text{constant}$$

where C represents the concentration of the solute in solvents I and II, and α the degree of dissociation or association in I and II respectively. Be expressing α in terms of the K_a and K_b of the sulfonamide and the pH of the gastric juice I found an analytical relationship between the equilibrium concentration ratios of these drugs and the pH of the gastric juice as follows:

Assume the existence of two aqueous well-buffered solutions of pH = P and pH = G respectively, separated by a

membrane (such as the gastric mucosa) which is freely permeable to the sulfonamide in the undissociated form. Let the total concentration of the sulfonamide and the concentration of any of its forms in either solution be much lower than saturation. The total concentration of the sulfonamide in either solution will be equal to the sum of the concentrations of the undissociated, basic, and acid forms.

(I) $$[T] = [U] + [B^+] + [A^-]$$

$[B^+]$ and $[A^-]$ can be expressed in terms of $[H^+]$, K_a and K_b

(II) $$[B^+] = \frac{K_b}{10^{-14}} [H^+] [U]$$

(III) $$[A^-] = \frac{K_a}{[H^+]} [U]$$

Substituting the values for $[B^+]$ and $[A^-]$ obtained from (II) and (III) in (I), and separating $[U]$, (IV) is obtained.

(IV) $$[T] = [U] \left(1 + \frac{K_b}{10^{-14}} [H^+] + \frac{K_a}{[H^+]} \right)$$

The concentration ratio between the solution with pH = G and the one with pH = P then becomes (V).

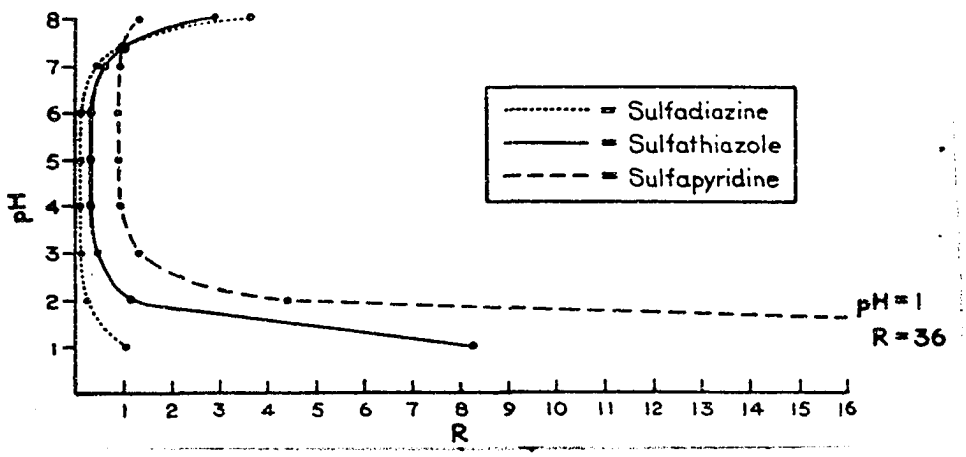
(V) $$R = \frac{[T]_G}{[T]_P} = \frac{[U]_G \left(1 + \frac{K_b}{10^{-14}} [H^+]_G + \frac{K_a}{[H^+]_G} \right)}{[U]_P \left(1 + \frac{K_b}{10^{-14}} [H^+]_P + \frac{K_a}{[H^+]_P} \right)}$$

Assume

(VI) $$[U]_G = [U]_P$$

Table VIII

Theoretical Concentration Ratios of Sulfapyridine, Sulfathiazole and Sulfadiazine at Different pH Values



Then:

$$(VII) \qquad R = \frac{\left(1 + \frac{K_b}{10^{-14}} [H^+]_G + \frac{K_a}{[H^+]_G}\right)}{\left(1 + \frac{K_b}{10^{-14}} [H^+]_P + \frac{K_a}{[H^+]_P}\right)}$$

The theoretical concentration ratios of sulfapyridine, sulfadiazine, and sulfathiazole, as obtained from equation (VII) for $[H^+]_G$ corresponding to pH values from 1 to 8 for gastric juice and for $[H^+]_P$ corresponding to the pH of plasma and tissue fluids (pH = 7.4), and using values of K_a and K_b for the sulfonamides as determined by Bell and Roblin[38] are plotted against the pH of the gastric juice in table VIII.*

On comparing the curve for sulfapyridine with the experimental data obtained for this drug (table VII) it becomes apparent that the experimentally determined concentration

---

*Identity of Symbols Used in Equations (I) to (VII).

$[T]$ = total concentration of sulfonamide

$[U]$ = concentration of undissociated sulfonamide

$[B^+]$ = concentration of basic form of sulfonamide

$[A^-]$ = concentration of acid form of sulfonamide

$[H^+]$ = concentration of hydrogen ion

K_a = acid dissociation constant of sulfonamide

K_b = base dissociation constant of sulfonamide

$[\,]_G$ = concentration in solution G

$[\,]_P$ = concentration in solution P

10^{-14} = the dissociation constant of H_2O

Values of K_a and K_b for Various Sulfonamides as Reported by Bell and Roblin.[38]

	K_a	K_b
Sulfapyridine	3.7×10^{-9}	3.8×10^{-12}
Sulfadiazine	3.3×10^{-7}	1.0×10^{-12}
Sulfathiazole	7.6×10^{-8}	2.3×10^{-12}

ratios of 4 to 10 at pH 1 to 2 are easily accounted for. The same is true for the concentration ratios at pH 7 to 8. However, at the intermediate pH range of 2 to 7 the experimentally obtained concentration ratios are appreciably larger than the theoretical values. This difference can be readily explained if one accepts the theory that gastric juice is a mixture of at least two secretions, one originating in the parietal cells with a pH of about 1, and the second originating from the remainder of the gastric mucosa with a pH of 7 to 8. The intermediate pH values and the corresponding concentration ratios are produced by mixtures of the two secretions.

For sulfadiazine the experimentally determined concentration ratios in men were generally around 0.5 and independent of the pH. Postulating again that gastric juice is a mixture of at least two different secretions with pH of about 1, and 7 to 8 respectively, one would expect a concentration ratio of about 1 from the calculated value, independent of the pH of the gastric juice. (The theoretical concentration ratio for sulfadiazine is about 1 for gastric secretion of pH 1 as well as for gastric secretion of pH 7 to 8). The fact that the experimentally determined concentration ratios were generally found to be around 0.5 may be atrributed to the low diffusion constant of sulfadiazine (ka = 0.252 (Davenport l.c.)).

For sulfathiazole, the calculated ratios fell between the values for sulfapyridine and sulfadiazine. Actually,

the concentration ratios found with this drug in both humans and dogs were appreciably lower (about 0.2). This again may be attributed to the low diffusion constant of sulfathiazole (ka = 0.175 (Davenport l.c.)), which is considerably smaller than the one of sulfapyridine (ka = 2.7 (Davenport l.c.)) or sulfadiazine.

The practical application of equation (VII) is thus limited by considerations involving the diffusion factor. In this connection it is interesting to note the striking parallellism between the order of magnitude of the diffusion factors of the sulfonamides and the degree to which they are bound to plasma proteins. The compounds with the lowest ka seem to be bound to plasma proteins to the greatest extent, and the ones with the greatest ka show the least plasma binding.(39)

On the other hand equation (VII) gains further theoretical support from chemical thermodynamics. The equation

$$\Delta F = nRT \ln \frac{f_2 c_2}{f_1 c_1}$$

gives the change of free energy when n mols of a solute are transferred from one solution where its activity is $f_1 c_1$ to another in which its activity is $f_2 c_2$. At equilibrium $\Delta F = 0$, i.e.

$$\frac{f_2 c_2}{f_1 c_1} = 1$$

Let f_G = activity coefficient of a sulfonamide in gastric juice

f_P = " " " " " plasma

c_G = concentration " " " " " gastric juice

c_P = " " " " " plasma

Then

(VIII) $$\Delta F = n\,RT\,\ln\frac{f_G c_G}{f_P c_P} = 0$$

and

(IX) $$\frac{f_G c_G}{f_P c_P} = 1$$

Davenport (l.c.) has determined the value of the ratio f_G/f_P for several sulfonamides, where f_G denotes the activity coefficient of the drug in 0.153 N HCl and f_P the activity in dog plasma. (Table IX).

Table IX

Values of f_G/f_P according to Davenport

Sulfonamide	f_G/f_P
Sulfapyridine	0.077
Sulfadiazine	0.931
Sulfathiazole	0.311

Using these values of Davenport to calculate c_G/c_P at equilibrium from equations (VIII) and (IX), and keeping in mind that equation (VII) was derived for and ideal system of dilute solutions, while the values of f_G/f_P were determined

on non-ideal systems, we find that the results obtained for c_G/c_P from (VIII) and (IX) are in general agreement with the values calculated from (VII) for $[H^+]_G = 10^{-1}$. (Table X)

Table X

Values for the Concentration Ratios Calculated from Equations VII and IX

Sulfonamide	VII	IX
Sulfapyridine	36	13
Sulfadiazine	1.0	1.07
Sulfathiazole	8.4	3.22

It appears likely that equations of the type (VII) hold not only for the excretion of sulfonamides into the gastric juice, but also for the distribution of these compounds between other body fluids and organs, for instance the distribution between plasma and red cells (whose pH is about 0.1 unit lower than that of plasma), excretion in bile, etc. Furthermore, it seems reasonable to expect that (VII) will be valid for compounds which resemble the sulfonamides from a physico-chemical point of view, such as certain dyes. Thus, the equation may become important in predicting the distribution of radioactive organic compounds in the body.

4) RADIOACTIVE COMPOUNDS IN THE DIAGNOSIS AND TREATMENT OF CANCER OF THE STOMACH

If radioactive compounds could be found which localize selectively in or close to a gastric tumor, they might well be useful in the diagnosis and treatment of cancer of the stomach. The terapeutic implications are obvious from the discussion on radiotherapy of cancer in Chapter 1.23. Their use as diagnostic tools is based on the methods for detection of radioactive substances. The distribution of radioactive compounds in the organism can be determined by means of Geiger-Mueller counters, electroscopes, or photography. Following the administration of a radioactive compound which has a tendency to localize selectively in a gastric neoplasm, there should be at the most a low concentration of radioactivity in a normal stomach. However, in the presence of a malignant growth, the concentration of the radioactive substance in the stomach should be increased greatly, since the compound accumulates in the tumor. There are several possibilities of detecting such an accumulation of radioactive material in the stomach in vivo. Externally it might be detectable by placing photographic films or Geiger-Mueller tubes in suitable positions on the patients chest, back and sides. However, since the radioactive isotopes which are at present available for this work are

chiefly short range beta-ray emitters, it will probably be more advantageous to measure the radioactivity in the stomach directly. 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. These devices can then be introduced into the patients stomach by the usual techniques and the radioactivity assayed in situ. It remains to find a radioactive substance which will localize selectively in a gastric neoplasm.

It is a well known fact that there are several naturally occuring elements and compounds which 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. Studies on leukemia and related diseases which make use of the uptake of radiophosphorus were mentioned previously. Radioiodine has been used extensively in the investigation of thyroid physiology. Hamilton and Soley 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.[40] Radioiodide appears promising in the treatment of a number of thyroid disorders, such as thyrocoxicosis. Unfortunately, in the presence of a neoplasm the thyroid often loses its ability to store iodine and for this reason there is little hope for radioiodine as a curative agent in these maladies. Iodine is also concen-

trated in the gastric contents and by the salivary glands. The mechanism of this phenomenon is poorly understood so far. I have made some studies on the excretion of radioiodine by the stomach and the salivary glands which shall be discussed later.

A number of synthetic organic compounds are known to localize with a high degree of selectivity in certain organs. We have discussed the concentration of sulfapyridine in the gastric contents. Other examples are the contrast media used in x-ray diagnosis for the visualization of the gall bladder or urinary tract, such as tetraiodophenolphthalein, 2-oxo-5-iodopyridine-N-acetate (Iopax), monoiodomethane sulfonate (Skiodan), and many others.

Numerous claims have been made to the effect that certain dyes are taken up by tumors with a high degree of selectivity. This discovery was essentially an outgrowth of the search for chemotherapeutic agents against cancer.(41) Some of these studies merit a short review.

Several papers by Roosen, Bernhardt, and others deal with an allegedly beneficial effect of Isamine Blue on certain tumors.(42) However, in the doses required the dye proved rather toxic. Furthermore it severely stained the skin of patients, the pigmentation lasting up to 9 years. On the other hand the results of the treatment were not very striking, and therefore it has been largely abandoned.

Warburgs studies on tumor glycolysis inspired a

search for compounds which would suppress tumor metabolism and thereby tumor growth without being toxic to the organism of the host. Karczag tested a number of dyes and other organic compounds for their ability to inhibit the glycolytic activity of zymase and whole yeast.(43) The compounds which inhibited zymase but not whole yeast Karczag assumed to be specific glycolysis inhibitors without being protoplasma poisons. He hoped that these compounds would show promise in cancer therapy. But animal experiments were disappointing. As a result of his studies Karczag emphasized the importance of taking into consideration the non specific properties of compounds, such as their solubility, dispersion, diffusibility, action on the reticulo-endothelial system etc. in evaluating their possible use as chemotherapeutic agents.

As early as 1909 Goldmann noted during studies concerning the physiological behavior of certain dyes that some of these compounds seemed to be concentrated by tumors.(44) These findings have been confirmed by numerous other observers.(45) In 1939 Duran-Reynals stated: "T-1824 and other poorly diffusible dyes injected intravenously localize selectively in spontaneous and transplantable tumors growing in mice, rabbits, and chickens."(46) Brunschwig, Schmitz and Clark confirmed the findings of Duran-Reynals in rats, and rabbits bearing benzpyrene sarcomas and carcinomas, benign adenofibromas, and fibrosarcomas of the gastric wall, and furthermore extended his observations to humans. Following

the intravenous injection of 30 to 100 mg Evans Blue they found that 20 of the 30 patients studied concentrated the dye selectively in and about the neoplastic foci. Three patients with carcinoma of the stomach were observed, and all three showed localization of the compound at the tumor site. The authors believed that they could satisfactorily explain the failure of dye localization in the 10 cases where no staining was seen (previous treatment with x-rays resulting in partial sclerosis of the lesions, etc.). "In some of the treated animal or human subjects benign neoplasms were also present, and these, in contrast to the malignant growth, did not show localization of the dye."(47)

In 1911 Wassermann and his associates tried to use dyes which were selectively taken up by tumors as a vehicle for substances which are of possible therapeutic value if they enter the tumor. They prepared Selenium-Eosin compounds which were claimed to be highly successful in the treatment of animal tumors. Eosin was used as a vehicle for the selenium, the latter being the active agent. Unfortunately the authors did not report how they prepared their compounds or their exact formulas.(48)

Some 30 years later, Zahland Cooper injected lithium salts of various dyes such as Trypan Blue, Pontamin Sky Blue 6B and Carminic Acid into tumor bearing mice. These compounds were to be used in the therapy of cancer in conjunction with slow neutrons.(49) Slow neutrons are biologically

rather inert. However, impact of a neutron on a Li atom leads to a nuclear reaction with a high release of energy:

$$_3Li^6 + _0n^1 \longrightarrow _2He^4 + _1H^3 + 4.6 \text{ mev}$$

The energetic particles thus formed have a very destructive effect on the tissues. The lithium salts of the dyes were supposed to localize selectively in the tumors, thereby producing a high concentration of lithium in the region in which the maximum radiation effect was desired. As had to be expected Zahl and Cooper found that the lithium salts of these dyes dissociate rapidly in the body, and that the lithium ions went their own way and did not deposit with the dye molecule in the tissues.

Tobin and Moore were the first to report a synthesis of radioactive dyes. They prepared radioactive dibrom-Trypan Blue and radioactive dibrom-Evans Blue by brominating o-tolidine with Br^{82} (half-life 34 hrs.) 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. Most of the radio-activity was found to be present in the liver, which was followed closely by the colon and feces. The average ratio between the radioactivity in the various tumors and the libers of the animals varied from 0.04 to 1.22, and was generally around 0.5, with other words, the liver took up about twice as much radioactivity as the tumor. While these

findings cast some doubt on the selectivity of the uptake of dyes by tumors "the significant fact remains, that the radioactive colloid permeates into and therefore radiates tumor tissue wherever this tissue may be, and no matter how widespread the metastases are."(50)

The work of Moore and Tobin showed clearly that determination of radioactive compounds in tissues with Geiger-Mueller counters is both simpler and more accurate than the chemical or tinctorial assays which had heretofore been employed in determining the localization of dyes. Since many dyes become bound to proteins and stain carbohydrates, such as cellulose, "fast", and because the amounts used for injection have to be comparatively small, it is very difficult to analyze the tissues for their dye content chemically. Most observers have merely estimated the distribution of the dyes by macroscopic or microscopic inspection and noted the intensity of the staining by a 1+, 2+, 3+, etc. designation. The reports in the literature and my own experience show that by this method agreement between macroscopic and microscopic findings is poor. Grossly, the presence of dye can be much more readily seen in a light colored tissue or organ, such as the cortex of the kidney or a mouse tumor, than in dark organs such as liver or spleen. On studying the toxicity of Iodo-Trypan Blue in dogs I found grossly that the cortex of the kidney was most intensely stained, while little discoloration was detectible in the spleen or liver. Under the

microscope the picture was just about reversed. Granules of the dye were most frequently observed in the liver and spleen, where they were stored in the reticulo-endothelial elements (Kupffer cells), but rarely in sections of the kidney. This discrepancy can readily be explained. The dye granules observed microscopically are due to an accumulation of the pigment by phagocitosis in form of small lumps; these lumps are then stored in the reticulo-endothelial system. They are readily visible under the microscope. The staining of the kidney however is evidently due to the presence of dye in a fine dispersed form, similar to that in which the dye is distributed in the blood stream. In this fine dispersion the dye can not be seen under the microscope. The following experiment may illustrate this statement: If 0.1 mg Evans Blue are added to 10 cc plasma, the plasma appears heavily stained blue. But if a drop of the stained plasma is now put under the microscope, no dye is visible.

So far, the selective uptake of dyes by malignant neoplasms can not be attributed to any specific characteristic of the tumors. It may be due to differences in the pH of the cells, or to increased capillary permeability. The latter is a phenomenon which occurs in many pathological conditions other than cancer, for instance in abscesses or burns.(51) The dyes which stain tumors have also been found to localize in abscesses. Furthermore, substances other than dyes were found to localize in tumors. Duran-Reynals (l.c.) for

instance reported accumulation of foreign sera in a number of animal neoplasms, as indicated by precipitin reactions. Dyes such as Evans Blue and Trypan Blue have been shown to combine with plasma proteins, particularly albumin, in quantitative proportions.(52) This would seem to indicate that the storage of these dyes in the tumors is due to an accumulation of certain proteins.

In view of the investigations discussed above it seemed worth while to prepare more radioactive dyes and related compounds in the hope that they might become useful in the diagnosis and treatment of malignant neoplasms, or that they might help to elucidate some of the theoretical and practical problems involved.

5) SYNTHESES OF ORGANIC COMPOUNDS

5.1) INTRODUCTION

In the search for radioactive compounds which will localize selectively in tumors, one would at first think of simply replacing stable elements in substances which had previously been reported to be taken up by neoplasms with their radioactive isotopes. Trypan Blue and Evans Blue are probably the two compounds whose uptake in tumors has been investigated most thoroughly. They contain the elements C, H, O, N, and S. Consequently one would be tempted to synthesize these compounds with radioactive isotopes of C, H, O, N, and S. This may indeed be possible in the not too distant future. However, when I began to study possibilities for the preparation of radioactive organic compounds some four years ago, it was not feasible to use the radioactive modifications of these elements. They either had a very weak radiation, which would make their assay in tissues difficult, or they were difficult to obtain from the cyclotron, or they had a short half-life, or any combination of the three.

It was for such reasons that Moore and Tobin chose Br^{82} to make their dyes radioactive. To them it had the adgantage of being readily synthesized into organic compounds. They were but a few miles from a cyclotron which

could supply them, and therefore the rather short half-life of Br^{82} (34 hrs.) was not too great a handicap despite the fact that the production costs of this isotope at the time were high.

To make radioactive organic compounds more generally available for tumor research it was desirable to employ an isotope which had a somewhat longer half-life with a radiation suitable for biological work, and one which could by synthesized into organic compounds with a firm linkage. I^{131} (half-life 8 days) met these specifications.

My choice of compounds was influenced by the work previously mentioned on various dyes, and by my experience with sulfonamides. Thus, I have attempted to make available a series of iodo- and radioiodo derivatives of mono- and bis-azo dyes and their intermediates, covering a range of molecular weights, solubilities, and degrees of acidity and basicity. My attention was focussed on substances which would contain iodine in a firm organic linkage, that is on compounds which would carry the iodine in an aromatic nucleus. I felt that this type of linkage may be expected to remain unaffected by metabolic processes within living organisms.

In developing the syntheses I have tried to take into consideration the practical needs of the investigators who may wish to use these radioiodo-compounds in physiological studies. Since I^{131} compounds can not be stored over any length of time, they have to be prepared shortly before they

are used. Time generally is an important factor once I^{131} enters into the synthesis. Therefore, I have paid attention to procedures in which the introduction of iodine constitutes the last step. The quantity by weight of radioactive material which is used in a physiological study is usually well below 1 gm, and for this reason I have studied some syntheses on a micro scale. In many clinical and physiological laboratories assistance of a highly skilled organic chemist is not available. Therefore I have tried to develop methods for radioiodination which would be simple enough to be carried out by personnel on a technician level.

The compounds reported may be divided into three groups: Intermediates; iodo-compounds, which were prepared as models; and radioiodo-compounds.

All the intermediates were azo dyes containing free amino or hydroxyl groups. They were obtained in the customary manner by coupling diazonium salts with phenols or amines in alkaline or slightly acid medium respectively. In this manner sulfanilic acid was coupled with phenol, resorcinol, and m-phenylenediamine, and o-tolidine with H-acid and Chicago acid. Hydroxy-Trypan Blue was prepared from Trypan Blue by diazotization of the amino groups of the dye followed by decomposition of the diazonium compound in an aqueous medium.

Three methods of iodination were employed: Elementary iodine in alkaline medium, iodine monochloride in acid medium, and the Sandmeyer reaction.

Heating of sulfanilic acid-azo-phenol in an hypoiodite solution gave only small amounts of the iodo compound. The procedure gave more complete iodination of azo dyes containing two hydroxy or amino groups in meta position to each other, and an unsubstituted ortho-para carbon in the benzene ring (sulfanilic acid-azo-resorcinol, sulfanilic acid-azo-m-phenolenediamine). It appeared that the azo linkage rendered amines and phenols less susceptible to iodination, possibly due to increased resonance in the molecule. Iodination with elementary iodine in an alkaline medium was highly successful in the case of 4-iodo-o-toluidine. This compound was obtained by refluxing a mixture of o-toluidine, iodine, calcium carbonate, water, and ether, followed by steam distillation.(53) p-Iodoaniline-azo-Nevile-Winther acid was obtained by coupling diazo-p-iodoaniline with the naphtholsulfonic acid.(54)

Sulfanilamide could be iodinated readily with iodine monochloride in acid aqueous solution, according to Scudi.(55) The iodosulfanilamide could be diazotized and coupled. Iodination of sulfapyridine with iodine monochloride in acid aqueous medium gave monoiodosulfapyridine in good yields. Sulfathiazole seemed to decompose when treated with iodine monochloride.

The preparation of iodo-H-acid from H-acid by the Sandmeyer reaction proved very difficult. Unless the reaction medium is kept rather strongly acid (pH 2), diazo-H-acid

has a strong tendency to couple with itself. On decomposition of the diazonium compound, most of the amino groups are replaced by hydroxyl groups. The 8-hydroxyl and 8-iodo derivatives have about the same solubility; both are very soluble in water. They can be precipitated from the aqueous phase with alcohol and ether. Once obtained, iodo-H-acid was found to couple readily with diazotized amines, such as diazosulfapyridine.

Surprisingly, replacement of the amino group by iodine through the Sandmeyer reaction is quite feasible when H-acid is part of an azo-compound, such as in Trypan Blue. Iodo-Trypan Blue can be prepared from Trypan Blue by the Sandmeyer reaction in satisfactory yields. The preparation of iodo-Neutral Red and iodo-Congo Red showed that it is possible to replace amino groups with iodine by the Sandmeyer reaction in a number of dyes.

Since iodination with iodomonochloride had been so successful, an attempt was made to prepare radioiodomonochloride from the sodium radioiodine obtained from M.I.T. by an exchange reaction. An aqueous solution of sodium radioiodide was shaken up with a solution of iodine monochloride in chloroform. It was found that an appreciable amount of radioactivity entered into the chloroform phase; however, under the conditions of the experiment the iodine monochloride decomposed, and further studies on the exchange reaction were abandoned.

Surprisingly enough I found that radioiodosulfanilamide and radioiodosulfapyridine can be obtained in good yields when acid aqueous solutions of the parent sulfonamide containing sodiumradioiodide are treated with iodine monochloride. The yieldswith respect to radioactivity are very satisfactory.

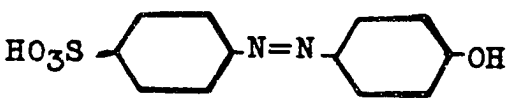
Radioiodo-Trypan Blue was synthesized from Trypan Blue by the Sandmeyer reaction. The yields with respect to radioactivity were best when an excess of the dye was employed. Accordingly, the final product was a mixture of hydroxy- and iodo-Trypan Blue. The toxicity of this mixture was studied in mice and dogs. It was found to be of the same order of magnitude as that of Evans Blue.(56) Radioiodotrypan Blue was administered to three patients. The details of these studies are reported in chapters 6) and 7).

5.2) EXPERIMENTAL

5.21) INTERMEDIATES

5.21.01) Sulfanilic Acid-Azo-Phenol

4-hydroxyazobenzene-4'-sulfonic acid



One tenth mol sulfanilic acid.$2H_2O$ (21 gm.) was dissolved in 25 cc 6N aq. NaOH and 100 cc water, and the pH checked for a value of 8 with indicator paper. To this solution were added 7 gm $NaNO_2$ (0.1 M) dissolved in 50 cc water. The alkaline sulfanilic acid - nitrite solution was dropped onto ice and 30 cc conc. hydrochloric acid (0.3 M) under effective stirring. After a short while a white precipitate appeared. Following the addition of the sulfanilic acid - nitrite solution stirring was continued for another 1/2 hr. at 0 - 3°C. During this time the diazotization mixture was checked repeatedly for the presence of excess nitrite and a pH 2. The 3.8 gm ammonium sulfamate (0.03 M) dissolved in a few cc water were added to destroy any excess NO_2^-. After stirring for another 1/2 hr. in the cold the mixture was considered ready for coupling.

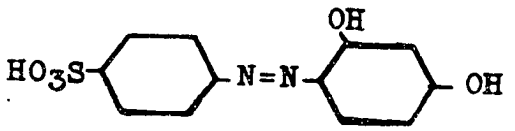
Ten gm (0.1 M) phenol were dissolved in 100 cc water containing 4 gm (0.1 M) NaOH and 21.2 gm (0.2M) Na_2CO_3.

The phenol solution was cooled by addition of a few pieces of ice. Then the diazotization mixture was added slowly and under effective stirring. Initially the coupling mixture assumed a bright red color. By the time coupling was complete a brownish yellow dye had precipitated from the solution. The mixture was stirred for several hours and during that time permitted to slowly warm up to room temperature, and then left standing over night. The next morning the precipitate was filtered off. The filter cake was made into a mush with 300 cc water, and this mush heated to boiling under stirring, whence it gave a dark reddish brown solution. On cooling a large mass of orange-yellow needles appeared. The recrystallized dye was filtered off, washed with cold water, and dried in vacuo over P_2O_5. The filtrate gave but a very slight turbidity with Ag^+, indicating that the pigment was practically free of Cl^-.

Yield: 24 gm bright golden-orange crystalline dye - 86% theory

5.21.02) Sulfanilic Acid-Azo-Resorcinol

2,4-dihydroxyazobenzene-4'-sulfonic acid



A mush was prepared by adding 50 cc 6N sodium hydroxide (0.3 mol) to 42 gm sulfanilic acid.$2H_2O$ and stirring it up. On addition of 200 cc water a slightly brown-yellow solution of pH 8 formed; to it were added 14 gm (0.2 mol) $NaNO_2$ dissolved in 100 cc water. The alkaline sulfanilic acid - nitrite solution was gradually poured into a mixture of ice and 60 cc conc. hydrochloric acid under effective stirring. The diazonium compound formed readily as a white precipitate. The mixture was stirred for 1/2 hr. at 0 to 5°C, whence it was ready for coupling.

Two tenth mol resorcinol (22 gm), 10.6 gm (0.1 mol) sodium carbonate, and 8.5 cc 6N sodium hydroxide (0.05 mol) were dissolved in 100 cc water. The diazo-sulfanilic acid suspension was gradually and under effective stirring added to the resorcinol solution. Toward the end of mixing there was gas development and an orange precipitate formed; the pH of the solution at the end of the mixing was 4. Solid sodium bicarbonate was added to the dye suspension under continued stirring until the pH of the mixture had risen to 8. It was

then stirred for 3 more hours at room temperature and then left standing for several days. The total volume was now about 1 liter, and the orange dye had settled. It was filtered off, washed with a small quantity of water, and dried in vacuo over P_2O_5.

Yield: 64 gm of a brick red, amorphous dye - 100% theory

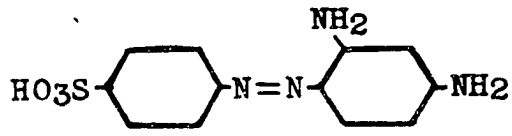
Sixty four gm of the crude dye was recrystallized from 400 cc water, and again dried in vacuo over P_2O_5.

Yield: 36 gm of a brick-red strongly electrophoric dye - 56% theory

The recrystallized dye was found to be free of chloride.

5.21.03) Sulfanilic Acid-Azo-m-Phenylenediamine

2,4-diaminoazobenzene-4'-sulfonic acid



One tenth mol sulfanilic acid was diazotized as described previously.

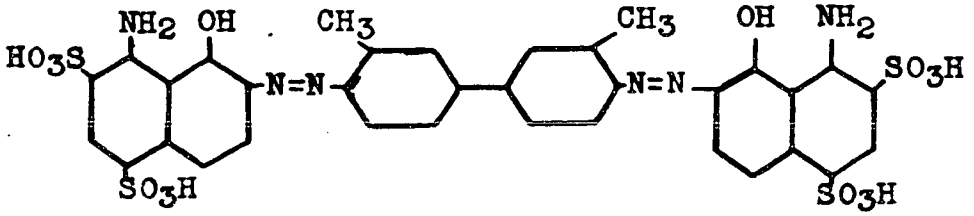
A solution of 22 gm (1.2 mol) m-phenylenediamine dihydrochloride in 100 cc water was prepared. It was cooled by addition of some ice, whence the diazosulfanilic acid suspension was added slowly and under effective stirring. The solution turned red. Upon addition of 20 gm crystalline sodium acetate a bright cinnabar red dye precipitated from the solution. The mixture was stirred in the cold for several more hours and then left standing at room temperature over night. The next day it was slowly and under stirring warmed up. As the temperature reached 75°C the color of the dye pigment turned from bright cinnabar red to dark red with a purple shade. The warm mixture was filtered and the residue washed with hot water until the filtrate was free of chloride ion. Then the dye was dried in vacuo over P_2O_5.

Yield: 30 gm bright red amorphous dye - 96% theory

The dye is only very slightly soluble in hot and cold water or dilute hydrochloric acid. It is readily soluble in dilute alkalies.

5.21.04) Evans Blue[57]

3,3'-dimethylbiphenyl-4,4'-bis(7''-azo-1''-amino-8''-hydroxy-2'',4''-naphthalenedisulfonic acid)



A suspension of 21.2 gm (0.1 mol) o-tolidine in 300 cc water containing 21 cc conc. HCl (0.4 mol) was diazotized by addition of 14 gm $NaNO_2$ (0.2 mol) in 40 cc water at 10 - 15°C under effective stirring. A fine yellow precipitate formed. The mixture was stirred for 1/2 hour at 10° to 15°C, whence excess nitrite was destroyed by addition or urea. The diazotization mixture was coupled at 18° with 82 gm (0.25 mol) Chicago acid dissolved in a solution of 32 gm (0.3 mol) Na_2CO_3 and 8 gm (0.2 mol) NaOH in 500 cc water. The mixture was stirred for several hours at room temperature and then left standing over night.

Fifteen gm Darco were then added and the mixture heated to 85° under effective stirring, and filtered while hot.

The dark purple filtrate was heated to 85° and 200 gm cryst. sodium acetate added. No precipitate formed and the filtrate was discarded.

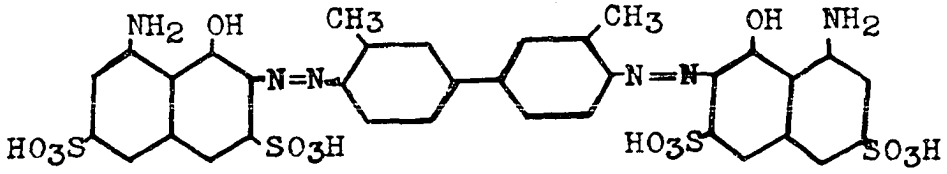
The filter cake was dissolved in 600 cc water at 85° and filtered quickly at that temperature. A residue,

apparently Darco, remained on the funnel; the filtrate was dark blue. The filtrate was again heated to 85°, the dye precipitated at that temperature by addition of 216 gm cryst. sodium acetate, and filtered off from the hot solution. The residue was reprecipitated from aqueous solutions with cryst. sodium acetate thrice more as described. The final press cake was dried on air at 110°C ground up and extracted three times with 95% ethyl alcohol under heating and stirring. The final alcoholic extract gave no more precipitate with a few drops conc. sulfuric acid, i.e. it was free of Na^+. The dye was dried in vacuo over P_2O_5.

Yield: 39 gm Evans Blue - 41% theory for the tetra sodium salt

5.21.05) Trypan Blue[57]

3,3'-dimethylbiphenyl-4,4'-bis(2''-azo-8''-amino 1''-hydroxy-3'',6''-naphthalenedisulfonic acid)



Thirteen gm o-toluidine dihydrochloride (46 millimol) were suspended in 150 cc water, the suspension acidified with 13 cc conc. hydrochloric acid, and diazotized at 10° to 15°C with 6 gm sodium nitrite (94 millimol) dissolved in 20 cc water. The diazotization mixture was stirred at 10° to 15°C for 1/2 hour. Then 1 gm urea was added to destroy excess nitrous acid; foaming was prevented by adding a few drops of n-octyl alcohol. The solution was now dark orange brown.

Fourty-one gm H-acid (0.11 mol) were dissolved in 370 cc water containing 4 gm NaOH and 17 gm sodium bicarbonate. The diazotization mixture was added slowly and under vigorous stirring to this H-acid solution at 10° to 15°C. The mixture turned dark purple. It was stirred at room temperature for two hours. The alkalinity of the mixture was checked frequently throughout the coupling process. The mixture was left standing at room temperature over night, whence the dye settled as a slimy precipitate. The dye was then filtered off and redissolved in 1000 cc water at 85° to 90°C. The solution was filtered while hot, the filtrate again heated

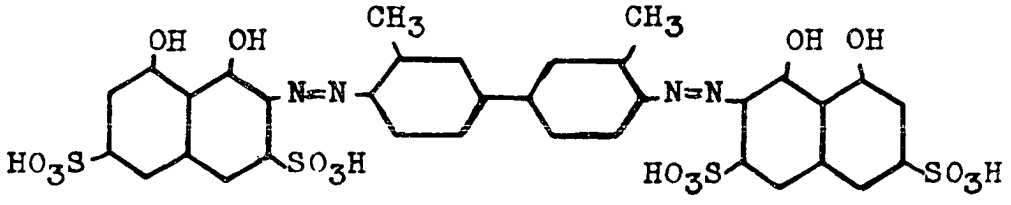
to 90°C and the dye precipitated with 300 gm cryst. sodium acetate. The mixture was cooled in an ice bath and the dye filtered off. The residue was again dissolved and reprecipitated as described above. The filtrate was now free of chloride ions. The residue was dissolved in 700 cc water at 85° to 90°C, the solution filtered hot, and the filtrate precipitated with 10 volumes of 95% ethyl alcohol. The precipitate was boiled up several times with 500 cc portions of 95% ethyl alcohol, until a sample of the supernatant no longer gave a precipitate with a few drops of conc. sulfuric acid, i.e. until all the Na^+ had been washed out. The dye was finally dried at 120°C for three days.

Yield: 18.5 gm Trypan Blue - 39% theory for the tetra sodium salt.

Analysis:	Found	Theory for tetra sodium salt
Ash (Na_2SO_4) %	28.8	29.6
N % (Tiedcke, Dumas)	8.5	8.7

5.21.06) Hydroxy-Trypan Blue

3,3'-dimethylbiphenyl-4,4'-bis(2''-azo-1'',8''-dihydroxy-3'',6''-naphthalenedisulfonic acid)



One hundredth mol Trypan Blue (9.6 gm) were dissolved in 100 cc water at 85° to 90°C under stirring. The hot solution was filtered, cooled back to room temperature and acidified with 10 cc conc. hydrochloric acid (0.1 mol HCl). The dye was then diazotized at 0°C by gradual addition of 1.7 gm (0.02 mol) $NaNO_2$ in 5 cc water. The color of the dye turned black green-grey. The diazotization mixture was stirred at 5° to 10°C for 30 minutes, and then left standing over night at room temperature, whence the color became purple. The solution was then heated in a boiling water bath (solution temperature 90°C) under stirring for one hour. Thirty gm cryst. sodium acetate were added to the hot solution, which was then cooled in an ice bath. Upon filtration of the cold solution no precipitate remained on the funnel, i.e. the sodium acetate had failed to break the solution. The dye was precipitated from the filtrate with 10 volumes (900 cc) 95% ethyl alcohol, and separated from the mother liquor by centrifuging. It was dried in the oven at 100°C, ground up, and then filtered hot. This extraction procedure was

repeated thrice more, whence the alcohol extract gave no more precipitate with conc. sulfuric acid, i.e. it was free from Na^+. The dye was then dried on the oven at 80°C.

Yield: 6 gm Hydroxy-Trypan Blue - 62% of theory

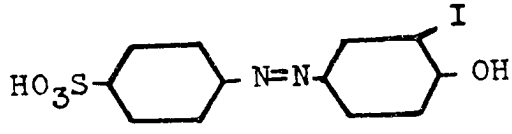
The aqueous solution of the Hydroxy-Trypan Blue was found to be red, while the Trypan Blue itself gave a blue solution in water.

5.22) IODO-COMPOUNDS

5.22.0) Iodination with Elementary Iodine in Alkaline Medium

5.22.01) Sulfanilic Acid-Azo-Iodophenol

4-hydroxy-3-iodoazobenzene-4'-sulfonic acid



Sulfanilic acid - phenol, 0.05 mol (15 gm) was dissolved in 50 cc water by addition of 10 cc 6N NaOH. The resulting solution was dark orange red.

Iodine (0.05 mol I_2 - 13 gm) was dissolved in 100 cc water containing 10.6 gm (0.1 mol) Na_2CO_3 at boiling temperature, giving a dark brown solution.

The hot alkaline iodine solution was added to the cold dye solution under stirring. A bright red precipitate formed; it was dissolved by addition of another 18 cc 6N NaOH, giving a clear brown-red solution. This solution was stirred at 90 to 95°C for 2 hours and then left standing over night at room temperature. Then 5 gm (0.05 mol) $NaHSO_3$ were added; the solution foamed slightly and a red precipitate appeared. The mixture was acidified to Congo-Red with 30 cc 6N HCl; there was heavy foaming, a strong SO_2 odor and an orange precipitate appeared. The precipitate was filtered off, washed with water, and dried in vacuo over P_2O_5.

Yield: 14 gm of an amorphous, orange-brown compound with an iodine or phenol like odor.

The above compound (14 gm) was recrystallized from 100 cc water, and again dried in vacuo over P_2O_5.

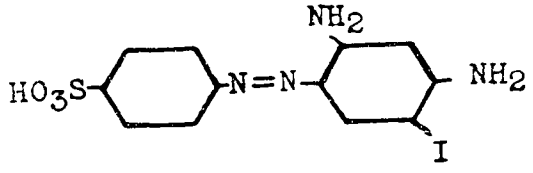
Yield: 6 gm of an amorphous, orange-yellow dye.

A sample of this compound dissolved in water acidulated slightly with HNO_3 gave no precipitate with Ag^+, that is it was free of halogen ion. A sample of the powder heated with a drop of concentrated sulfuric acid developed iodine vapors.

Analysis: (Parr Bomb)	Found	Calculated
% I	3.61	31.5
% S	10.4	7.95

5.22.02) Sulfanilic Acid-Azo-Iodo-m-Phenylenediamine

2,4-diamino-5-iodoazobenzene-4'-sulfonic acid



Sulfanilic acid - m-phenylenediamine (9.4 gm - 0.033 mol) was dissolved in 400 cc water containing 16 gm (0.15 mol) Na_2CO_3 at 55°C. (When a sample of the solution was cooled, an orange-red crystalline precipitate appeared.) The solution was dark orange-red-brown. Iodine (8.5 gm - 0.033 mol I_2) was added and the mixture stirred at 50 to 60°C for 1 hour. It was then filtered rapidly while hot. There was no significant residue. Under stirring 3.5 gm (0.033 mol $NaHSO_3$) were added to the filtrate, and it was subsequently acidified gradually with 20 cc conc. HCl. Initially a black-brown and coarse precipitate appeared, toward the end the precipitate was red and fine. The mixture was left standing over night. The precipitate then seemed to consist of two components, one made up of fine red particles, the other one made up of coarse dark red-brown particles.

The residue was filtered off, In the process of filtration the two compounds could be separated fairly well. Most of the fine precipitate (A) was collected on one Buchner funnel and most of the coarse precipitate on another (B). Both (A) and (B) were washed with water and dried in vacuo

over P_2O_5.

Yield: (A) 2.5 gm, purple-black amorphous compound

(B) 6 gm, purple-black amorphous compound

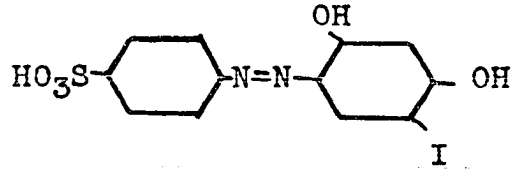
(B) was only slightly soluble in hot and cold water, ethyl alcohol, and glacial acetic acid. The aqueous extract gave no precipitate with Ag^+, indicating the absence of halogen ions.

A sample of dry (B) on heating with a drop of concentrated sulfuric acid developed strong iodine vapors.

Analysis: (B)(Parr Bomb)	Found	Calculated
% I	21.6	30.4
% S	8.3	7.3

5.22.03) Sulfanilic Acid-Azo-Iodoresorcinol

2,4-dihydroxy-5-iodoazobenzene-4'-sulfonic acid



Sulfanilic acid-resorcinol (11 gm - 0.04 mol) and 11 gm (0.1 mol) Na_2CO_3 were dissolved in 100 cc water, giving a dark brown solution. To this solution were added 13 gm (0.05 mol I_2) iodine and the mixture stirred at 60°C for 1 hour. It was then cooled to room temperature, whence 2.4 gm $NaHSO_3$ (0.025 mol) and 8 cc conc. HCl were added, rendering the solution acid to Congo Red and producing a thick red precipitate. The precipitate was filtered off and washed with water; it appeared quite soluble. The dye was dried in vacuo over P_2O_5.

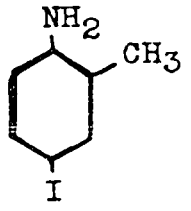
Yield: 10 gm of a red, amorphous, strongly electrophoric compound.

A sample of the dye dissolved very readily in a small quantity of water. A slightly acidulated hot solution on cooling formed a red jelly. The aqueous solution gave no precipitate with Ag^+, that is it was free of halogen ions. The dye was found to be soluble in hot ethyl alcohol and glacial acetic acid; it was insoluble in Dioxane and acetone.

Analysis: (Parr Bomb)	Found	Calculated
% I	23.4	30.2
% S	7.2	7.6

5.22.04) Iodo-o-Toluidine (58)

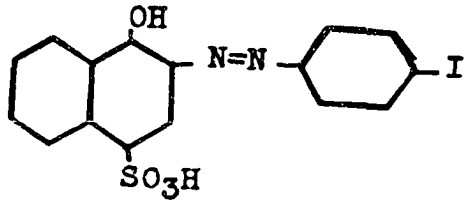
4-iodo-2-methylaniline



Two tenth mol iodine (50 gm) and 60 gm precipitated $CaCO_3$ (0.6 mol) were ground together and refluxed with 30 gm (0.28 mol) o-toluidine, 75 cc ether, and 45 cc water for 1 hour. The mixture was then steam distilled. White crystals slightly contaminated with iodine separated from the distillate. The crude iodo-compound thus obtained was filtered off and recrystallized from a solution of 200 cc ethyl alcohol and 20 cc conc. HCl. A pure white compound was obtained.
Yield: 12.5 gm - 23% theory for $C_7H_8NI.HCl$

5.22.05) p-Iodoaniline-Azo-Nevile-Winther Acid.

2-(4'-iodophenylazo)-1-hydroxynaphthalene-4-sulfonic acid



One tenth mol p-iodoaniline (21.9 gm) was dissolved in 800 cc water containing 40 cc conc. HCl (0.4 mol HCl) at about 45°C. This solution was added slowly and under effective stirring to a mixture of ice, 6.9 gm (0.1 mol) sodium nitrite, and a few cc of water and thus diazotized at 0 to 5°C.

One tenth mole Nevile-Winther salt (24.6 gm), 24.6 gm (0.1 mol) Na_2CO_3, and 20 cc 6N NaOH were dissolved in 200 cc water and the diazonium salt solution gradually and under effective stirring added in the cold. A bright red dye began to precipitate immediately toward the end of mixing gas development began. The coupling mixture was left standing over night and then filtered. The filtrate had a pH of about 9. The residue was washed with water, and then stirred up with 1000 cc water and heated to 100°C under continued stirring. Most of the dye did not go into solution. The suspension filtered poorly. The residue was washed with water and dried in vacuo over P_2O_5.

Yield: 15 gm of a bright red compound

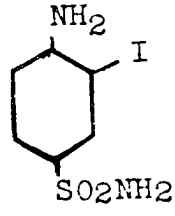
Analysis:	Found	Calculated
% I	31	27
% S	5.7	6.7

The dye was found to be but slightly soluble in hot and cold water, aqueous sodium bicarbonate, and ethyl alcohol. It was practically insoluble in dilute hydrochloric and sulfuric acid. Anaqueous dilute sulfuric acid extract gave no precipitate with Ag^+, indicating that the compound was free of halogen ions. The dye decomposed on heating with dilute nitric acid.

5.22.1) Iodination with Iodine Monochloride in Acid Medium

5.22.11) Monoiodosulfanilamide[59]

4-amino-3-iodobenzenesulfonamide



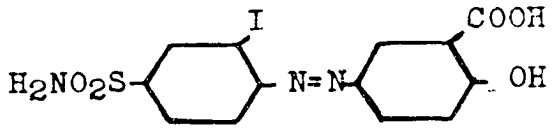
One tenth mol sulfanilamide (17.2 gm) were dissolved in 1000 cc water acidified with 30 cc conc. hydrochloric acid at 35°C. The solution was cooled to 10°C and 15.2 gm (0.1 mol) ICl added under stirring. A brown precipitate formed. The mixture was left standing for two hours at room temperature then the residue was filtered off. It was boiled up with 800 cc water containing 25.2 gm (0.2 mol) sodium sulfite, whence the precipitate dissolved almost completely. The hot solution was quickly filtered by suction. On cooling monoiodosulfanilamide crystalized from the mother liquor in long, white needles. The crystals were filtered off, washed with water until the wash water no longer decolorized N/10 KI - iodine solution, and then with ether; they were dried over P_2O_5 in vacuo. A sample of the substance dissolved in dilute nitric acid gave no precipitate with Ag^+, indicating absence of iodide and chloride.

Yield: 15 gm 50% based on sulfanilamide theory

Analysis: (Parr Bomb)	Found	Theory
% S	10.8	10.7
% I	43.5	42.6

5.22.12) Monoiodosulfanilamide-Azo-Salicylic Acid

2-iodo-4-sulfamylazobenzene-4'-hydroxy-3'-carboxylic acid



Six gm (0.02 mol) monoiodosulfanilamide were dissolved in 50 cc 1/2N aq. sodium hydroxide containing 1.4gm (0.02 mol) sodium nitrite. This solution was dropped from a burette into 200 cc water containing 10 cc conc. hydrochloric acid, HCl, and pinch of sodium nitrite under stirring. The compound diazotized readily under these conditions (temperature: 0 - 3°C).

Two hundredth mol salicylic acid (2.8 gm) and 4 gm sodium carbonate were dissolved in 50 cc water. The diazotization mixture was slowly added to this solution under vigorous stirring at 0 to 5°C. The solution turned red. It was left standing at room temperature for 12 hours. Then the dye was precipitated with 10 cc conc. hydrochloric acid; it formed a red clump of rubber like consistency. The dye clump was washed with water and dried in vacuo over P_2O_5.

The free acid is almost insoluble in boiling water; a water extract gave no precipitate with Ag^+, indicating the absence of iodide and chloride. The compound is readily

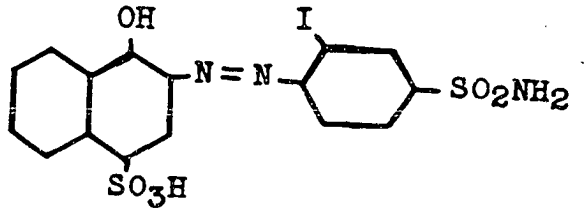
soluble in 95% ethyl alcohol.

Yield: 6 gm 62% theory

Analysis: (Parr Bomb)	Found	Theory
% S	8.8	7.2
% I	36.4	28.4

5.22.13) Monoiodosulfanilamide-Azo-Nevile-Winther Acid

2-(2'-iodo-4'-sulfamylphenylazo)-1-hydroxynaphthalene-4-sulfonic acid



A solution of 12 gm (0.04 mol) monoiodosulfanilamide, 4 gm (0.1 mol) NaOH, and 2.8 gm (0.4 mol) $NaNO_2$ in 100 cc water was prepared. This solution was slowly (within 1/4 hour) dropped into 400 cc water containing 25 cc conc. HCl (0.25 mol), maintained at 0-3° C, under effective stirring. A fine orange-yellow suspension formed. Stirring was continued for 1/2 hour following the addition of the sulfonamide solution, whence the mixture was ready for coupling.

A solution of 10 gm (0.04 mol) Nevile-Winther salt, 16 gm (0.15 mol) Na_2CO_3 and 1 gm NaOH was prepared and cooled to 0-3° C. The cold diazotization mixture was slowly added to this alkaline solution.under effective stirring. A dark red solution formed initially; finally a cinnabar red dye was obtained as a fine precipitate. The mixture was stirred at room temperature for several hours and then left standing in the laboratory for about a day. It was then warmed on a water bath, whence the dye went into solution. The solution was evaporated to about 200 cc, acidified with 20 cc glacial

acetic acid, and cooled in an ice bath. The dark vine-red precipitate thus obtained was readily filterable.

The dye residue was warmed on a boiling water bath with 250 cc 95% ethyl alcohol under stirring, the mixture cooled in the ice box, and the dye filtered off, washed with 95% EtOH and dried in vacuo over P_2O_5.

Yield: 7 gm - 32% theory

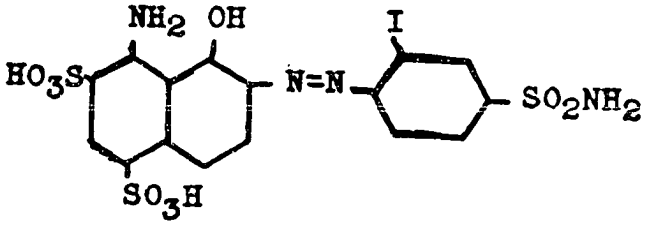
The 7 gm Monoiodosulfanilamide-Azo-Nevile-Winther Acid were stirred up on a boiling water bath with 50 cc 95% ethyl alcohol for about 1 hour. Not nearly all the precipitate was dissolved. The mixture cooled in the ice box, and was then filtered. The filtrate gave no precipitate with a few drops conc. sulfuric acid, i.e. it was free of Na^+. The residue was dried in vacuo over P_2O_5.

Yield: 5 gm Monoiodosulfanilamide-Azo-Nevile-Winther Acid

Analysis: (Parr Bomb)	Found	Theory Monosodium Salt
I %	22.9	22.9
S %	10.7	11.5

5.22.14) Monoiodosulfanilamide-Azo-Chicago Acid

7-(2'-iodo-4'-sulfamylphenylazo)-1-amino-8-hydroxy-5,7-naphthalenedisulfonic acid



Twelve gm monoiodosulfanilamide (0.04 mol) was dissolved in a solution of 4 gm NaOH (0.1 mol) and 2.8 gm (0.04 mol) $NaNO_2$ in 60 cc water. This solution was slowly dropped from a burette into 200 cc water containing 25 cc conc. hydrochloric acid (0.25 mol HCl), and a pinch of $NaNO_2$. The diazotization was carried out at 0-3° under effective stirring. The solution turned yellow and part of the diazonium compound separated as a fine precipitate. The mixture was stirred in the cold for another 1/4 hour, whence 6 gm urea were added to destroy excess nitrite.

The diazotization mixture was coupled with a solution of 13 gm (0.04 mol) Chicago acid, 18 gm (0.17 mol) Na_2CO_3 and 1 gm NaOH in 50 cc water at 0-3° under effective stirring. A thick, dark vine-red colloidal solution formed. It was evaporated on a steam bath to about 80 cc and acidified with 25 glacial acetic acid, whence a chocolate brown dye precipitated. The mixture was digested on a hot water bath for a short time, and then filtered while warm. The precipitate

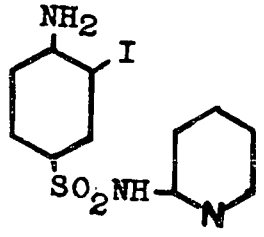
was resuspended in a few cc water and 3 vol. 95% ethyl alcohol added, and left standing over night. The dye was then filtered off, washed with 75% ethyl alcohol and dried in vacuo over P_2O_5.

Yield: 2.5 gm Monoiodosulfanilamide-Azo-Chicago Acid - 10% theory for the tetra sodium salt

Analysis: (Parr Bomb)	Found	Theory for Tetrasodium salt
I %	17.8	17.8
S %	13.0	13.4

5.22.15) Monoiodosulfapyridine

4-amino-3-iodo-N^1(2'-pyridyl)-benzenesulfonamide



One tenth mol sodium-sulfapyridine.H_2O (28.9 gm) was dissolved in 700 cc water containing 35 cc concentrated hydrochloric acid. The solution was cooled to 10°C and 16 gm (0.1 mol) iodine monochloride added under stirring; a beige precipitate formed presently. The mixture was stirred for one hour at room temperature, whence the precipitate was filtered off. It was boiled up with 2-1/2 l water containing 25.2 gm (0.1 mol) sodium sulfite; most of the precipitate did not go in solution. The hot mixture was filtered quickly. The residue was dissolved in 100 cc boiling glacial acetic acid and 5 gm Darco added to the solution. The glacial acetic acid solution was filtered hot. On cooling large crystals separated from the brown filtrate. The crystals were filtered off and recrystallized from 30 cc glacial acetic acid. The final crop was washed with cold glacial acetic acid and water, and dried in vacuo over P_2O_5.

A sample of the iodosulfapyridine thus obtained was dissolved in dilute nitric acid. It gave no precipitate

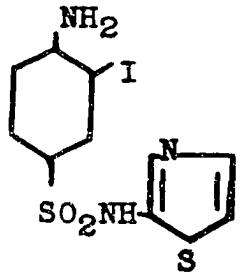
with Ag^+, indicating absence of iodide.

Yield: 8 gm 21% monoiodosulfapyridine as a white, amorphous powder.

Analysis: (Parr Bomb)	Found	Theory
S %	8.62	8.5
I %	34.5	34.0

5.22.16) Monoiodosulfathiazole

4-amino-3-iodo-N^1(2'-thiazolyl)-benzenesulfonamide



One tenth mol sodium sulfathiazole (29.5 gm) was dissolved in 200 cc water containing 35 cc conc. hydrochloric acid. The solution was cooled to 0-3°C, and 16 gm (0.1 mol) iodine monochloride added under stirring. A fine white precipitate was formed. The mixture was stirred for 1/2 hour at 10°C, during which time the precipitate turned beige. The precipitate was filtered off. It rapidly turned dark brown when exposed to air. It was stirred up with aq. sodium sulfite; this treatment changed the color to a light yellow. The sodium sulfite was removed by washing with water and the compound dried in vacuo over phosphorous pentoxide; on drying it again turned dark brown and began to smell strongly of free iodine.

Attempts were made to recrystallize the compound from glacial acetic acid and 95% ethyl alcohol. It was not soluble in either; on heating the mixtures the compound formed a tarry lump. The compound was soluble in 1N aq. sodium hydroxide.

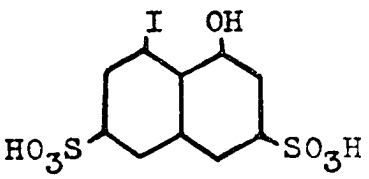
The compound was then suspended in 200 cc ethyl ether and left standing for 2 days. Then it was filtered off, washed with ether, and dried in vacuo over P_2O_5. The product thus obtained had an orange-beige color.

Analysis: (Parr Bomb)	Found	Theory
% S	21.2	16.8
% I	9.16	33.4

5.22.2) Iodination by the Sandmeyer Reaction

5.22.21) Iodo-H-Acid

1-hydroxy-8-iodo-3,6-naphthalenedisulfonic acid



Principle of Synthesis:

Iodo-H-acid was synthesized from H-acid (1-hydroxy-8-amino-3,6-naphthalene disulfonic acid). The H-acid was suspended in water, the suspension acidified with sulfuric acid and diazotized with sodium nitrite. The diazo compound was readily obtained as a bright yellow, crystalline precipitate. It was filtered off and decomposed in the presence of iodide. The iodo-H-acid was finally isolated by precipitation with alcohol and ether.

Example:

Twelve gms H-acid (purified) (0.04 mol) was suspended in 400 cc water, the suspension acidified with 20 cc conc. H_2SO_4 and diazotized at 0-5°C with 56 cc of a 4% solution of $NaNO_2$ in water. The nitrite was added rapidly and an excess of NO_2^- and acidity to Congo Red maintained during the diazotization. The mixture was stirred at 0-5°C for 1/2 hour. The diazo-H-acid separated from the solution in

bright yellow, crystalline needles. When diazotization was completed excess nitrite was destroyed with urea, and the diazo-H-acid filtered off by suction.

The diazo-H-acid was immediately suspended in a solution of 40 gm sodium iodide in 400 cc water; the pH of this suspension was 1.0-1.5. The suspension was kept at 37° to 38°C for 48 hours. By this time the yellow diazo-H-acid had disappeared and the solution was dark red-brown. The solution was heated to boiling, cooled back to room temperature, and the pH adjusted to 7-8 with 20% aqueous sodium hydroxide.

The iodo-H-acid was precipitated from the aqueous solution with 6 volumes of 95% ethyl alcohol and 7 volumes of ethyl ether. After the voluminous precipitate had settled the larger part of the supernatant was decanted and the rest centrifuged. The residue was redissolved in 20 cc water and again precipitated with 120 cc 95% ethyl alcohol, and 140 cc ethyl ether. After the precipitate had settled it was separated from the liquid phase by centrifuging, and finally dried in vacuo at 90-100°C.

The disodium salt of iodo-H-acid thus obtained was a beige-white amorphous and hard mass. It was very soluble in water; the aqueous solution gave no precipitate with Ag^+. It coupled readily in alkaline, but not in acid solution. The substance darkens on standing; it has a characteristic odor. Yield: 6 gms

Analysis:		Found	Theory
Ash (Na_2SO_4)	%	26	30
I (Tiedcke)	%	21.8	26.8
S	%	11.8	13.6

Discussion:

Technical H-acid (Eastman) was the raw material for the synthesis. It was purified prior to use as follows:

50 gm crude H-acid were suspended in 800 cc water and 10 gm Darco added to the suspension. The mixture was heated to boiling under continuous stirring. When the H-acid had dissolved completely, the hot solution was quickly filtered by suction. On cooling H-acid reprecipitated from the green filtrate. The H-acid filtered off and redissolved under boiling in 500 cc water; the solution was acidified with a few drops sulfuric acid and filtered quickly by suction while hot. On cooling to room temperature H-acid again precipitated. It was filtered off (leaving a light green filtrate), washed with water and acetone and air dried. Thus 30 gm (60%) of H-acid was obtained. It was a white, slightly greenish tinged, glittering mass of asbestos like appearance and consistency. Further recrystallizations (up to 8 times) did not improve the appearance of the H-acid. Also, the mother liquor retained a greyish-green tinge. This discoloration seems to be due to oxidation of H-acid.

The diazotization of H-acid with sodium nitrite at 0-5°C in an acid aqueous suspension proceeds smoothly and

quantitatively. The solution may be acidified with hydrochloric, sulfuric, or acetic acid. The diazonium compound separates from the reaction mixture as golden yellow crystals. It may be filtered off and is quite stable. It has a tendency to form a purple pigment, probably due to coupling between diazo-H-acid molecules. This decomposition is furthered by excess of water and higher pH values (7) of the medium.

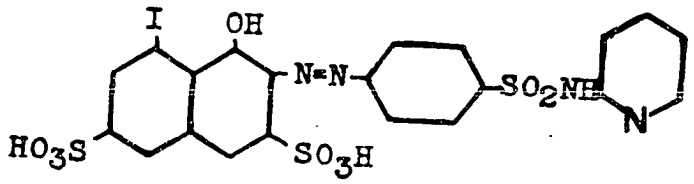
Contrary to expectations, replacement of the diazonium group by iodine was found to be rather difficult. When the diazo-H-acid is decomposed in an iodide containing solution, free elementary iodine is formed initially. The free iodine disappears after a while. The diazo-H-acid has a strong tendency to couple with itself; unless the solution is sufficiently acid a purple pigment (mentioned above) is formed which is very soluble in water, but not as soluble as iodo-H-acid in ethyl alcohol. Decomposition of the diazo-H-acid in I^- containing solution of different concentrations was tried over a temperature range from about 20° to 100°C, within varying time interludes, with HI and NaI as sources of I^-, and with $CuSO_4$ and "Natuskupfer" (both of the latter forming white CuI) as catalysts. The method outlined above in detail was found superior to all other modifications.

Great difficulty was also encountered in precipitating the iodo-H-acid from its aqueous solution. The compound is unusually soluble in water. Ethyl alcohol will not precipitate it from water. It is possible to

precipitate the compound from concentrated aqueous solutions with HCl gas; but the product thus obtained is very unstable, giving off elementary iodine. Precipitation with EtOH and ethyl ether was chosen because they give a stable product and can readily be removed by drying in vacuo at moderate temperatures.

5.22.22) Iodo-H-Acid-Azo-Sulfapyridine

2-(N^1-2''-pyridyl-4'-sulfamylphenylazo)-1-hydroxy-8-iodo-3,6-naphthalenedisulfonic acid



Nine millimol sodium sulfapyridine.H_2O (2.6 gm) was dissolved in 50 cc water and acidified with 5 cc concentrated hydrochloric acid. The solution was diazotized at 0-5°C with 6.9 cc 10% aqueous sodium nitrite (10 millimol). Excess sodium was finally destroyed with urea.

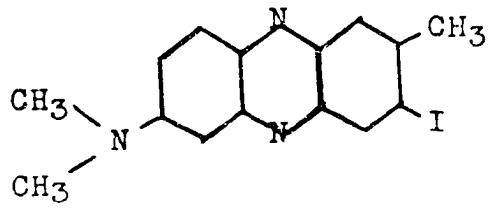
Eight millimol Iodo-H-acid (3.7 gm disodium salt) were dissolved in 10 cc 20% aqueous sodium hydroxide, and this solution added to the diazotization mixture. The solution turned red. It was stirred for 1 hour in the cold and then slowly heated to 80°C in a water bath. After the solution had cooled back to room temperature, the dye was precipitated with concentrated hydrochloric acid and separated by centrifuging. The residue was washed, under stirring and centrifuging, with aqueous hydrochloric acid (about 7%), and finally with 95% ethyl alcohol. The red dye thus obtained was dried in vacuo at 95°C.

Yield: 2.73 gm 50% theory

Analysis:	Found (Tiedcke)	Theory
N %	9.20	8.1
S %	11.8	13.9
I %	16.8	18.4

5.22.23) Iodo-Neutral Red

7-dimethylamino-3-iodo-2-methylphenazine

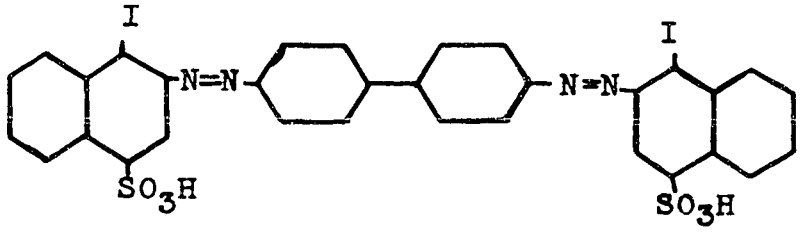


One millimol Neutral Red (288 mg) was dissolved in 15 cc water. The solution was acidified with 1 cc conc. sulfuric acid and the compound diazotized at 0° to 5°C by gradual addition of 69 mg sodium nitrite (1 millimol) dissolved in 1 cc water. The diazotization mixture was stirred in the cold for 1/2 hour. Then 1 gm urea was added to destroy excess nitrous acid, rendering the potassium-iodide-starch reaction negative. Now, 300 mg (2 millimol) NaI dissolved in a few cc water were added, and the mixture left standing at room temperature for 48 hours. The iodocompound was precipitated with 250 cc ethyl alcohol and 500 cc ethyl ether and separated by centrifugation. It was dried in vacuo.
Yield: 276 mg of a purple-black amorphous compound - 67% theory

The compound was only sparingly soluble in water; the solution was dark purple. On addition of alkali the color turned bright yellow. The aqueous solution gave no precipitate with Ag^+, indicating that the compound was free of halogen ions. A sample of the compound on heating with a drop of concentrated sulfuric acid developed iodine vapors.

5.22.24) Iodo-Congo Red

biphenyl-4,4'bis(2-azo-1-iodo-4-naphthalenesulfonic acid)



Eight millimol Congo Red (5.6 gm) was suspended in 60 cc water, acidified with 4 cc (72 millimol) concentrated sulfuric acid and cooled to 0-5°C. The Congo-Red was diazotized at that temperature by dropwise addition of 1.12 gm (16 millimol) sodium nitrite in 5 cc water. After all the sodium nitrite had been added, the solution was stirred in the cold for another 20 minutes. Then 2 gm urea were added. After another 10 minutes of stirring the HI-starch reaction for free nitrous acid had become negative, 4.8 gm (32 millimol) sodium iodide in 10 cc water were now added. The solution was warmed up slowly under continuous stirring. A brownish cast appeared; this color shade disappeared again after the mixture had been stirred at 21°C for about 10 minutes; however an odor reminiscent of iodine remained for some time. At 21°C gas development started. The temperature was raised slowly (within 3/4 hour) to 82°C and held at that temperature for 1/4 hour. By then the gas development had stopped, and the solution was dark purple. The dye was precipitated with 10 cc concentrated hydrochloric acid and separated from the

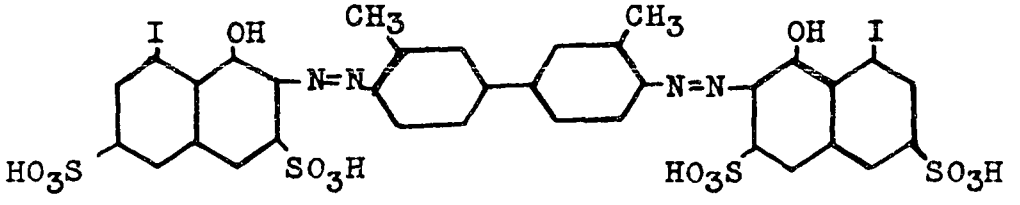
mother liquor by centrifuging. The yellow supernatant was discarded. The black purple residue was suspended in 100 cc water reprecipitated with 10 cc concentrated hydrochloric acid and again centrifuged. Finally the dye was washed several times with acetone under stirring and centrifuging, and air dried.

Yield: 1.5 gm Iodo-Congo Red

Analysis: (Parr Bomb)	Found	Theory for free acid
I %	9.5	29.1
S %	7.7	7.3

5.22.25) Iodo-Trypan Blue

3,3'-dimethylbiphenyl-4,4'-bis(2-azo-1-hydroxy-8-iodo-3,6-naphthalenesidulfonic acid)



a) From o-tolidine and iodo-H-acid:

A suspension of o-tolidine (1.59 gm = 7.5 millimol) in 20 cc water was prepared. It was acidified with 4 cc concentrated hydrochloric acid, and the amine diazotized with 2.5 cc 40% sodium nitrite solution at 0 - 5°C. The diazotization mixture was stirred for 15 minutes in the presence of excess nitrite at that temperature. Then urea was added until the potassium iodide - starch reaction for free nitrous acid became negative. The solution containing the diazotolidine was added slowly and under effective stirring to a solution of 12 millimol iodo-H-acid (5.8 gm disodium salt) in 30 cc water containing 1.6 gm NaOH. The dark purple solution thus obtained was stirred for 1/2 hour at room temperature, and then heated to 85°C in a water bath. The pH of the solution was adjusted to a value of 6 to 7 with glacial acetic acid (glass electrode). Then the dye was precipitated with 800 cc 95% ethyl alcohol and separated from the mother liquor by centrifuging. The residue was washed with portions

95% ethyl alcohol under warming, stirring, and centrifuging, until a sample of the supernatant no longer gave a turbidity on addition of a drop of concentrated sulfuric acid, i.e. until the dye was free of Na^+. The dark purple pigment was then dried in vacuo at 90 to 100°C.

Yield: 3.11 gm dye.

Analysis:	Found	Theory for tetrasodium salt
Ash (Na_2SO_4) %	24.0	24.0
I (Tiedcke) %	14.8	21.5
S (Tiedcke) %	9.9	10.8

b) From Trypan Blue by the Sandmeyer reaction:

Eight millimol Trypan Blue (7.68 gm) were dissolved in 50 cc water, acidified with 5 cc (0.11 mol) glacial acetic acid and cooled to 0 - 5°C. The trypan blue was diazotized at that temperature by dropwise addition of a total of 1.12 gm (16 millimol) sodium nitrite in 5 cc water under vigorous stirring. After all the sodium nitrite had been added, the solution was stirred in the cold for another 15 minutes. In the course of the diazotization it had formed into a thick black-grey-green mass. Finally, 2 gm urea were added to destroy excess nitrous acid.* After another 10 minutes of stirring 4.8 gms (32 millimol) sodium iodide in 10 cc water

*The HI-starch reaction will be slightly positive even after excess nitrous acid has been destroyed with urea. The diazo-trypan blue apparently oxidizes iodide to iodine.

were added. Brownish vapors which blued HI-starch paper were observed (obviously elementary iodine); these vapors disappeared again after a short while. The mixture was then slowly (within 1 hour) and under continuous stirring heated to 85°C, and kept at that temperature for 1/2 hour. It changed into a dark purple solution. The dye was precipitated (from 70 cc aqueous solution) with 280 cc 95% ethyl alcohol and 350 cc ethyl ether. It was separated from the mother liquor by centrifuging, washed repeatedly with 95% EtOH, and finally with acetone. It was first air dried in a funnel on a water pump, and then in vacuo over sodium hydroxide pellets.

Yield: 6 gm Iodo-Trypan Blue

Analysis: (Parr Bomb)	Found	Theory for tetrasodium salt
I %	16.5	21.5
S %	9.9	10.8

5.23) RADIOIODO-COMPOUNDS

5.23.01) Exchange of Radioiodine between an Aqueous Solution of Sodium radioiodide and a Solution of Iodine Monochloride Chloroform.

An aqueous solution of NaI containing 150 mg NaI in 100 cc solution (A), ($10^{-4}M_{NaI}$/liter), and a chloroform solution containing 8 gm ICl in 50 cc ($1M_{ICl}$/liter) (B) were prepared.

Into each of three glass stoppered 15 cc test tubes were pipetted 5 cc of solution (A) and 5 cc of solution (B). (Tubes No. 1, 3, 5). Into 3 similar test tubes were pipetted 5 cc of solution (A) and 5 cc chloroform. (Tubes No. 2, 4, 6).

The tubes were then treated as follows:

Tube No.	Treatment
1	Shaken on shaking machine for 1/2 hour.
2	Shaken by hand for a few minutes
3	Shaken on machine for 1/2 hour. Left standing for 4 days.
4	Shaken on machine for 1/2 hour. Left standing for 4 days.
5	Shaken on machine for 1-1/2 hour. Left standing for 4 days.
6	Shaken on machine for 1-1/2 hour. Left standing for 4 days.

The iodinemonochloride - chloroform solution was initially dark brown. During the shaking process it turned purple and some elementary iodine precipitated, i.e. the ICl was decomposed. The aqueous phase became light brown.

All tubes were centrifuged shortly before the layers were transferred for activity measurement, in order to assure complete separation of the phases.

Then two 2 cc aliquots from each phase of every tube were pipetted into a 4 cc screw cap vial. The activity of these vials was then measured on the Geiger-Mueller counter.

Results of activity measurements:

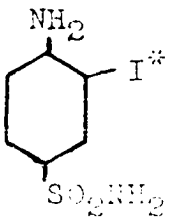
Sample		Corr. Aver. C/min./cc	Sum	Activity in aq. / Activity in $CHCl_3$
1. aq.	Machine shaken 1/2 hour	45	70	1.8
1. ICl		25		
2. aq.	Hand shaken for a few minutes	124	124	
2. $CHCl_3$		0		
3. aq.	Machine shaken 1/2 hour	61	91	2.0
3. ICl	stand 4 days	30		
4. aq.	Machine shaken 1/2 hour	119	119	
4. $CHCl_3$	stand 4 days	0		
5. aq.	Machine shaken 1-1/2 hour. Stand 4 days	63	89	2.3
5. ICl		26		
6. aq.	Machine skaken 1-1/2 hour. Stand 4 days	98	98	
6. $CHCl_3$		0		
Standard		102		

From the above table it is apparent that the sum of the C/min./cc for the aqueous phase + C/min./cc for the chloroform - iodinemonochloride phase is somewhat smaller than the sum of the C/min./cc for the aqueous + chloroform blank phases. It seems that some of the radioiodine had located in the solid iodine phase which had formed, but had not been transferred into the vial.

The ratio of activity in the aqueous phase/activity in the ICl-$CHCl_3$ phase seems to increase slightly with time, which is surprising. One would rather have expected a decrease with time. Whether or not any significance may be attributed to the slight increase of the above ratio can not be determined from the data at hand.

5.23.02) Monoradioiodosulfanilamide

4-amino-3-radioiodobenzenesulfonamide



To 8.5 gm (0.05 mol) sulfanilamide were added 15 cc concentrated hydrochloric acid and then 500 cc water; on stirring at room temperature all the sulfanilamide dissolved.

The solution was cooled to 10°C by addition of a few pieces of ice. Twenty-five cc NaI* solution (I^{131} -, half-life 8 d) were added and then under effective stirring 7.6 gm ICl (0.05 mol); a beige precipitate appeared. The mixture was stirred at room temperature for 1 hour and then left standing in the laboratory for 2 days. The beige brown precipitate settled; the supernatant was clear and slightly yellow.

The precipitate was filtered off, washed with water and then with ethyl ether, and dried in vacuo over P_2O_5.

(I) Yield: 11 gm Monoradioiodosulfanilamide (beige, amorphous) - 74% theory be weight

Nine gm of the crude monoradioiodosulfanilamide were dissolved in 600 cc water acidified with 2 cc 6N hydrochloric acid and containing 10.4 gm (0.1 mol) $NaHSO_3$ at

boiling temperature, giving a yellow solution. The hot solution was quickly filtered by suction (leaving no residue) and then cooled at about 3°C for 3 hours. A white, crystalline precipitate separated. It was filtered off, washed with water until the washings no longer decolorized aqueous potassium iodide-iodine solution, and dried in vacuo over P_2O_5.

(II) Yield: 7 gm Monoradioiodosulfanilamide (cryst., white) - 64% by weight of I, 47% by weight of sulfanilamide.

Radioactivity measurements were made with a commercial type Geiger-Mueller counter (Cyclotron Specialties Co.). The samples (about 1 gm) were placed in 4 cc screw cap glass vials and the vials put under the bell-type counter tube. All assays were run in duplicate.

Results of activity measurements, in counts as recorded on the 1:8 scaling circuit of the instrument used and with the geometry employed, corrected for 2/1/46:

Counts added (in 25 cc NaI* solution, containing 0.1 millimol NaI/liter) 2450 C/M

Specific activity of crude monoradioiodosulfanilamide 184 C/M/gm

Total activity in 11 gm crude monoradioiodosulfanilamide (I) 2024 C/M

% Counts recovered for crude monoradioiodosulfanilamide 82

Specific activity of recryst. monoradioiodosulfanilamide 205 C/M/gm

Total activity in 7 gm recryst. monoradioiodosulfanilamide (II)

1435 C/M

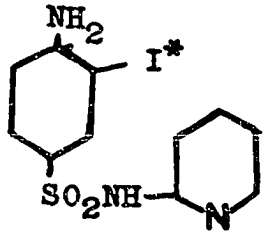
% counts recovered for recryst. monoradioiodosulfanilamide

58

Approximate specific activity of recryst. monoradioiodosulfanilamide 1 micro-c/gm

5.23.03) Monoradioiodosulfapyridine

4-amino-3-radioiodo-N^1(2'-pyridyl)-benzenesulfonamide



One tenth mol sodiumsulfapyridine.H_2O (28.9 gm) was dissolved in 1 liter of water containing 35 cc concentrated hydrochloric acid and sodium radioiodide. The solution was cooled to 10°C by addition of some ice, whence 16 gm (0.1 mol) iodine monochloride were added dropwise under effective stirring. A beige precipitate formed. The mixture was stirred at room temperature for one hour. Then the precipitate was filtered off. The filtrate was greenish yellow. A small amount of white precipitate separated after it had stood for a while. The residue was washed with water and dried in vacuo over P_2O_5.

Yield: 38 gm Radioiodosulfapyridine, crude (dark beige, amorphous) - 100% by weight

Thirty-four gm of the crude compound were suspended in 1 liter of water containing 21 gm (0.2 mol) $NaHSO_3$ and 10 cc dilute (6N) HCl and heated to boiling under stirring. The solid phase remained largely undissolved. The mixture was cooled in an ice bath. Then the residue was filtered

off, washed with water until the washings no longer decolorized a dilute aqueous iodine solution, and finally with ether. The substance was dried in vacuo over P_2O_5.

Yield: 31 gm Radioiodosulfapyridine (light ocher, amorphous) - 91% by weight

Results of activity measurements, in counts as recorded on the 1:8 scaling circuit of the instrument used (cyclotron specialties counter) and with the geometry employed, corrected for 2/26/26:

Counts added (in 50 cc NaI* solution, containing 0.001 mg I)
4875 C/M

Specific activity of crude radioiodosulfapyridine 100 C/M/gm

Total activity in 38 gm crude radioiodosulfapyridine
3800 C/M

% Counts recovered for crude radioiodosulfapyridine 78

Specific activity of purified radioiodosulfapyridine
101 C/M/gm

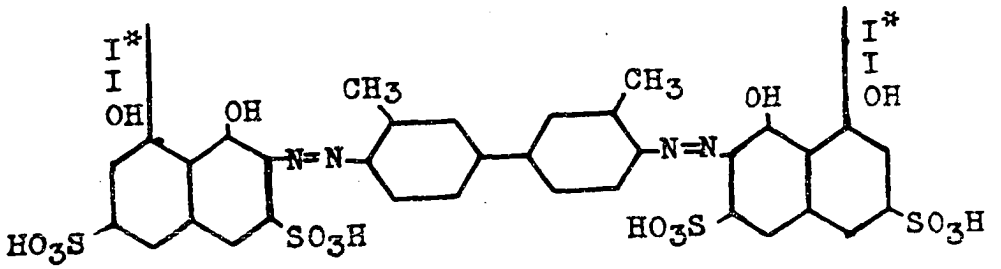
Total activity in 31 gm purified radioiodosulfapyridine
3131 C/M

% Counts recovered for purified radioiodosulfapyridine
based on counts added 64
based on crude radioiodosulfapyridine 101

Note:

To obtain good yields it is essential that the iodination be carried out in a dilute solution and that the iodine monochloride is added slowly and under effective stirring.

5.23.04) Radioiodo-Trypan Blue



One third millimol of Trypan Blue (320 mg tetra sodium salt) were dissolved in 5 cc water. The dark blue-purple solution was acidified with 1 cc 5 Molar sulfuric acid (5 millimol H_2SO_4) and diazotized at 0 to 5°C by gradual addition of 2/3 millimol (46 mg) $NaNO_2$ dissolved in 1 cc water. The solution changed its color to green-grey. The diazotization mixture was stirred for 1/2 hour in the cold, whence 100 mg ammonium sulfamate were added, and stirring continued for another 1/4 hour. Then 1 cc of radioiodide-solution of the desired activity and 15 mg (0.1 millimol) sodium iodide were added. The solution was now slowly warmed to 50°C and stirred at that temperature for 6 hours. The color changed to reddish-purple. Finally the solution was heated to 96 to 100°C and kept at that temperature for another hour. After it had been cooled back to room temperature, the dye was precipitated with 50 cc ethyl alcohol and 100 cc peroxide free ether, and separated from the mother liquor by centrifuging. The compound was then dried, taken up in a suitable amount of water and the pH adjusted

to 7.0 to 7.5 with sodium hydroxide and sodium bicarbonate. A glass electrode was used to check the pH values. For injection, the dye solution was further diluted with physiological saline, filtered, and sterilized.

Note:

The procedure outlined above was adopted for the preparation of radioiodo-Trypan Blue for physiological studies on the basis of some 60 runs in which the conditions for the decomposition of the diazo-Trypan Blue, such as temperature, time, amount of NaI, etc., had been varied. The dye obtained is a mixture of hydroxy-Trypan Blue, iodo-Trypan Blue, and radioiodo-Trypan Blue. The yields with respect to radioactivity are in the neighborhood of 70%.

6) STUDIES CONCERNING THE TOXICITY OF RADIOIODO-TRYPAN BLUE

6.1) STUDIES ON MICE

The toxicity of non radioactive iodo-Trypan Blue was investigated on a total of 110 mice. It was felt that the toxicity of radioiodo-Trypan Blue due to the organic part of the molecule should be of about the same order of magnitude as that of the non radioactive compound. As to possible toxic effects due to the radiation of radioiodine, it was known that humans could tolerate a single dose of 1,000 microc. without any untoward effects.

The results obtained with iodo-Trypan Blue are given in Table IX. None of the mice showed any ill effects from s.c. injections of up to 50 mg/kg of the compound. In larger doses iodo-Trypan Blue was found to be a slow acting poison. Toxic symptoms appeared usually on the 5th to 6th day following the injection. If the animals appeared healthy on the tenth day, it seemed reasonable to assume that they would show no ill effects from the injection later on.

The toxicity of Trypan Blue was studied on 54 mice for purposes of comparison. The results are shown in Table X. Iodo-Trypan Blue and Trypan Blue were found to have about the same toxicity.

Table IX

Toxicity of Iodo-Trypan Blue in Mice

Dose mg. Iodo-Trypan Blue/Mouse (25 gm)	Route of Injection	No. of Mice Used	No. of Mice Dead Within 10 days
1.0	s.c.	10	0
2.5	"	20	1
3.5	"	10	4
5.0	"	33	27
10	"	20	20
15	"	7	6
50	"	6	5
2.0	i.v.	1	1
20	"	3	3

Table X

Toxicity of Trypan Blue in Mice

Dose mg. Trypan Blue/Mouse (25 gm)	Route of Injection	No. of Mice Used	No. of Mice Dead Within 10 days
2.5	s.c.	10	3
3.0	"	9	0
5.0	"	20	13
25	"	10	10
50	"	3	3
20	i.v.	2	2

6.2) STUDIES ON DOGS

The toxicity of radioiodo-Trypan Blue was studied on 15 dogs. The samples of radioiod-Trypan Blue were only very slightly radioactive. The total amount of radiation administered never exceeded 10 microc. per animal. The compound was given intravenously in saline. Doses ranged from 5 to 110 mg/kg. All animals were observed for not less than 3 and not more than 38 days. Four dogs died in the course of the study apparently from the effects of the dye. The others were killed at various time intervals by i.v. injection of 20 cc ether. All animals were autopsied and their tissues and organs studied macroscopically and microscopically.*

Details of the experiments and their results are given in Table XI. The dogs tolerated doses up to 50 mg/kg without untoward symptoms. One dog, which received 68 mg/kg, showed a heavy reaction immediately after the dye had been injected. He died about 12 hours later. Several dogs showed significant prolongation of the prothrombin time shortly after the dye had been administered. The prothrombin time returned to its normal value within 24 hours following the injection.

Autopsy revealed the kidneys to be stained blue grossly in all the animals. On microscopic examination the

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*I am greatly indebted to Dr. Ralph Fuller of the Department of Pathology for preparation and reading of the slides.

Table XI

Studies on Dogs

Identity	Radioiodo-Trypan Blue mg/kg	Death	Macroscopic	
#788 m. 9 kg	71	38 d after inj. 20 cc ether i.v.	Kidneys: Cortex strongly blue Medulla slightly blue Otherwise normal	Studie stomac Dye de Otherw
#789 m. 8.1 kg	64	38 d after inj. 20 cc ether i.v.	Kidneys: Cortex strongly blue Otherwise normal	Studie stomac liver Dye de Otherw
#790 f. 15 kg	38	37 d after inj. 20 cc ether i.v.	Kidneys: Cortex blue Otherwise normal	Studie liver, creas, Urinar and ch tion, cells Scars deposi Dye de
#740 m. 22.5 kg	26	6 d after inj. 20 cc ether	Skin slightly blue. Ribs blueish cast. Intestine blue. Kidneys very strongly blue throughout. All blood vessels strongly purple blue.	Studied epidid pancrea Dye de

Table XI

Studies on Dogs

scopic	Microscopic	Remarks
tex strongly tly blue mal	Studied: Lung, spleen, kidneys stomach, intestine, liver. Dye deposit: Liver 1+ Spleen 2+ Otherwise normal	Prothrombin time: 24 hrs. after inj. 9 sec.; plasma strongly purple 48 hrs. after inj. 8 sec.; plasma purple
tex strongly mal	Studied: Lung, spleen, kidneys, stomach, intestine, pancreas, liver Dye deposit: Liver 3+ Spleen 4+ Otherwise normal	Prothrombin time: 24 hrs. after inj. 9 sec.; plasma strongly purple 48 hrs. after inj. 7 sec.; plasma purple
tex blue mal	Studied: Lung, spleen, kidney, liver, stomach, intestine, pancreas, urinary bladder Urinary bladder shows acute and chronic inflammatory reaction, but the inflammatory cells contained no pigment. Scars in the kidney show no dye deposit. Dye deposit: Liver 1+ Spleen 2+	Prothrombin time: Before inj. 9 sec. 10' after inj. 8 sec.; plasma strongly purple 24 hrs. after inj. 8 sec. 6 d hfter inj. 8 sec.
blue. Ribs Intestine s very throughout. sels strongly	Studied: Liver, festis, epididymus, stomach, kidneys, pancreas, lungs Dye deposit: Liver 2+	Prothrombin time: Before inj. 9 sec. 10' after inj. 14 sec.; plasma strongly blue 24 hrs. after inj. 9 sec. 6 d after inj. 9 sec.

#791 m. 16.5 kg	68	Spontaneous 12 hrs. after inj.	Lungs: In parts very strongly blue. Liver: Strongly blue on capsule. Kidneys: Heavily stained throughout. Pancreas: Very heavily stained purple-blue. Heart muscles: stained. Testicles: purple-blue	Studied: g.-i.-trac spinal cor All tissue mortem cha epididymit Dye deposi
#793 f. 8.3 kg	58	Spontaneous 8 d after inj.	Kidneys: stained throughout Had hemorrhage from nose and lungs	Studied: oviduct, m kidney, lu bladder. Advanced p No dye dep
#754 f. 10.3 kg	68	16 d after inj. 20 cc ether i.v.	Kidneys strongly blue, particularly cortex	Studied: stomach, i bladder, u propria of Dye deposi
#755 f. 6.5 kg	77	16 d after inj. 20 cc ether iv.	Kidneys very strongly blue, particularly cortex	Studied: stomach, i pancreas, ovary, adr Dye deposi
#757 f. 11 kg	110	Spontaneous 5 d after inj.	Kidneys: capsule blue. Had abscess below the right forehand, the inflamed area was heavily stained blue in sharp contrast to the surrounding normal tissue.	Studied: kidney, st lymphnode. Dye deposi

arts very strong- iver: Strongly sule. Kidneys: ined throughout. Very heavily ple-blue. Heart tained. purple-blue	Studied: Liver, heart, muscle, g.-i.-tract, kidney, lung, spinal cord, testis, epidymus All tissues show advanced post mortem changes. Chronic epididymitis. Dye deposit: Liver 1+	Prothrombin time: Before inj. 8 sec. 10' after inj. 26 sec. Had heavy reaction immediately after inj.
ained throughout age from nose	Studied: Liver, spinal cord, oviduct, myocardium, pancreas, kidney, lung, stomach, uruary bladder. Advanced post mortem changes No dye deposit noted.	Prothrombin time: Before inj. 8 sec. 10' after inj. 24 sec.
ongly blue, y cortex	Studied: Lung, spleen, kidney, stomach, intestine, lymph, node, bladder, uterus, liver, lamina propria of endometrium Dye deposit: Liver 1+ Spleen 2+ Lymphnode 2+ Lamina propria of endometrium	Prothrombin time: Before inj. 10 sec. 10' after inj. 20 sec.
y strongly blue, y cortex	Studied: Lung, spleen, kidney, stomach, intestine, bronchius, pancreas, oviduct, oesophagus, ovary, adrenal Dye deposit: Spleen 2+	Prothrombin time: Before inj. 10 sec. 10' after inj. 20 sec.
apsule blue. below the and, the in- was heavily e in sharp con- e surrounding ue.	Studied: Lung, spleen, liver, kidney, stomach, intestine, lymphnode. Dye deposit: Liver trace Spleen 3+ Lymphnode 2+	Prothrombin time: Before inj. 8 sec. 10' after inj. 12 sec.

#765 m. 10.2 kg	23	20 d after inj. 20 cc ether i.v.	Kidneys: Intestine	Cortex blue c large number of worms	died: stomach, Dye depos
#775 f. 7.6 kg	24	20 d after inj. 20 cc ether i.v.	Kidneys:	Cortex blue	died: stomach, liver. Dye depos
#781 f. 12.7 kg	8	21 d after inj. 20 cc ether i.v.	Kidneys:	Cortex slightly blue	Studied: stomach, bladder, Dye depos Phagocyti propria c cus of action dy (app withou
#763 m. 11.8 kg	9	21 d after inj. 20 cc ether i.v.	Kidneys:	Cortex blue	tudied: omach, pancreas, Dye depos
#500 m. 19.1 kg	5	3 d after inj. 20 cc ether i.v.	One kidney removed, other one wrapped in silk sebaceous cyst on tail		Studied: stomach, pancreas, ode, aor yst, epi e depos Phagocyti ells col perimenta in focus flammat ebaccous

ox blue ge number	died: Lung, spleen, kidney, stomach, intestine, testis, liver Dye deposit: Liver 1+ Spleen 2+	Prothrombin time: 10' after inj. 9 sec.
ox blue	died: Lung, spleen, kidney, stomach, intestine, uterus, liver. Dye deposit: Liver 2+ Spleen 3+	Prothrombin time: Before inj. 8 sec. 10' after inj. 10 sec.
ox slightly	Studied: Lung, spleen, kidney, stomach, intestine, uterus, bladder, liver. Dye deposit: Liver 1+ Spleen 2+ Phagocytized pigment in lamina propria of endometrium. ous of chronic inflammatory action surrounding a foreign dy (apparently a small worm) without dye.	Prothrombin time: Before inj. 10 sec. 10' after inj. 9 sec.
ox blue	tudied: Lung, spleen, kidney, omach, intestine, bladder, pancreas, liver Dye deposit: Spleen trace	Prothrombin time: Before inj. 9 sec. 10' after inj. 9 sec.
ved, other silk on tail	Studied: Lung, spleen, kidney, stomach, intestine, gall bladder, ancreas, liver, heart, lymph de, aorta, testis, sebaccous rst, epididymus, lymph node. e deposit: Liver 1+ Spleen 2+ Lumph node 2+ Phagocytized dye in endothelial cells collected about kidney (experimental perinephritis; no dye in focus of acute suppurative inflammation of a lymph node or in sebaccous cyst.)	Hypertensive dog; courtesy of Cardiac Lab.

#1795 f. 12.6 kg	1st d Radioiodo-Trypan Blue 25 mg/kg 8th d Trypan Blue 8 mg/kg 12th d Trypan Blue 8 mg/kg	Pneumonia Spontaneous 14th d	Skin: Strongly blue Lungs: Pneumonia Kidneys: Cortex strongly blue Ribs: blueish Stomach: mucosa shows hypertrophy and an ulcer of 10 mm ø near site of inj. of histamine and turpentine; also an abscess. Acute peritonitis localized in the region of the experimentally produced lesion in the stomach.	Fatty Dye de No dye
#761 m. 14 kg	Congo Red 36 mg/kg	28 d after inj. 20 cc ether i.v.	Normal	Studie intest liver, No dye
#758 m. 9.5 kg	Congo Red 53 mg/kg	28 d after inj. 20 cc ether i.v.	Normal	Studie stomac testis. Dye dep
#756 m. 14.2 kg	Neoprontosil 36 mg/kg	12 d after inj. 20 cc ether i.v.	Normal	Studie testis, kidney, Dye dep Pigment cells a inflamm in kidn

blue a x strongly a shows an ulcer of site of inj. d turpentine; . Acute alized in the experi- ced lesion .	Fatty infiltration of the liver. deposit: Liver 1+ Spleen 3+ Lymph node 1+ No dye about the gastric lesion.	1st d. Operation (Nembutal) 0.4 cc turpentine in gastric wall 5th d. Gastroscopy (Nembutal) 7th d. " " 12th d. Operation (Nembutal) 0.5 mg histamine and 1.0 cc turpentine inj. into gastric wall.
	Studied: Lung, spleen, stomach, intestine, lymph node, bladder, liver, kidney. No dye found.	Prothrombin time: Before inj. 8 sec. 10' after inj. 20 sec.
	Studied: Lung, spleen, kidney, stomach, intestine, liver, testis. Dye deposit: Liver 1+ Spleen 2+	Prothrombin time: Before inj. 8 sec. 10' after inj. 12 sec. Had red stools
	Studied: Lymph node, bladder, testis, liver, lung, spleen, kidney, stomach, intestine Dye deposit: Liver 1+ Spleen 2+ Lymph node 2+ Pigment containing endothelial cells about foci of chronic inflammatory reaction scattered in kidney.	Prothrombin time: Before inj. 8 sec. 10' after inj. 9 sec.

#764 f. 9.9 kg	Neoprontosil 50 mg/kg	12 d after inj. 20 cc ether i.v.	Normal		Studi [illegible]a ovidu Dye d Focus react in re [illegible]ei ment lesio
#760 f. 8.8 kg	Trypan Blue 57 mg/kg	28 d after inj. 20 cc ether i.v.	Mucous membranes Skin Intestine Spleen Ribs Stomach Heart Uterus Urinary bladder Urine Kidneys: strongly blue Lungs: normal	Blue	Studi lung, intes Dye d

	...died: Lung, spleen, kidney, ...mach, intestine, bladder, oviduct, liver. Dye deposit: Liver trace Spleen 1+ Focus of acute inflammatory reaction in a leuticular gland in relation to a culcified foreign body in stomach, no pigment containing cells about this lesion.	Prothrombin time: Before inj. 8 sec. 10' after inj. 9 sec.
mbranes	Studied: Bladder, uterus, liver, lung, spleen, kidney, stomach, intestine	Prothrombin time: Before inj. 7 sec. 10' after inj. 14 sec.
Blue	Dye deposit: Spleen 1+	
ladder		
strongly blue rmal		

dye was generally found in the liver and spleen. One dog had an abscess below the right forepaw. On gross examination the inflamed area showed strong blue pigmentation in sharp contrast to the surrounding normal tissues.

As controls, two dogs were injected each with Trypan Blue, Congo Red, and Neoprontosil in comparable doses. Post mortem examination showed Trypan Blue to be distributed similarly to the radioiodo compound. The tissues and organs of the animals injected with Congo Red and Neoprontosil showed no discoloration grossly. However the dyes were found microscopically in the liver and spleen. Both Trypan Blue and Congo Red gave rise to prolonged prothrombin times shortly after the injection; Neoprontosil was without effect on the prothrombin time.

7) ADMINISTRATION OF RADIODO-TRYPAN BLUE TO PATIENTS

1st Patient:

E.W. No. 172,803 m. Age 81.

Diagnosis: Scirrhus adenocarcinoma of the stomach.

On September 1, 1943, the patient received an intravenous injection of about 25 mg radioiodo-Trypan Blue of low specific activity in 20 cc saline. The injection was tolerated well. There were no reactions, and no changes in the patients blood pressure or pulse rate. A sample of the patient's serum 1 hour after the injection showed no discoloration.

The patient received further injections of the compound as follows:

September 2, 50 mg radioiodo-Trypan Blue of low specific activity.

September 4, 100 mg radioiodo-Trypan Blue of low specific activity.

September 7, 200 mg radioiodo-Trypan Blue of low specific activity.

September 10, 300 mg radioiod-Trypan Blue of low specific activity.

All these injections were tolerated well and there were no signs of any toxic effects.

The patient expired on September 11.

Exerpt from autopsy report (Dr. Fuller): "No localization of dyestuff can be grossly or microscopically detected. The toxic changes do not differ from those ordinarily seen in cases of advanced malignant neoplastic disease."

Data on the radioactivity measurements for this study were lost at M.I.T.

2nd Patient:

L.McC. No. 187,345 m. Age 39

Diagnosis: Malignant teratoma.

On October 15, 1943, the patient received 100 mg radioiodo-Trypan Blue of low specific activity in saline i.v. The injection was tolerated without any untoward effects. The patient expired on October 29.

Data on the radioactivity measurements for this study were lost at M.I.T.

3rd Patient:

E.K. No. 175,747 m. Age 62

Diagnosis: Carcinoma of the rectum.

The patient received radioiodo-Trypan Blue as follows:

Date	mg Radioiodo-Trypan Blue	Activity microc.
October 19, 1943	60	5
October 20, 1943	120	10

(cont)

Date	mg Radioiodo-Trypan Blue	Activity microc.
October 21, 1943	240	15
October 23, 1943	480	25
October 27, 1943	360	1

All injections were tolerated well and no symptoms of toxicity were observed.

The patient expired on October 29, 1943.

Note from autopsy report (Dr. Fuller): "No abnormal pigmentation of the tissues was observed grossly. Microscopically, no phagocytosis of exogenous dye-stuff was shown by cells of the reticulo-endothelial system, inflammatory cells or tumor cells."

Radioactivity assay of tissues (M.I.T.):

No activity could be detected in a biopsy specimen of the tumor. Activity of tissues obtained on autopsy: Specimens of the spleen showed a very slight activity; activity in the kidney cortex was a little greater. No activity was detected in other tissues and organs.

8) THE EXCRETION OF RADIO-IODIDE BY THE HUMAN STOMACH

8.1) INTRODUCTION

In 1928 Heilmeyer and Sturm brought out the fact that iodide ion, following its administration intravenously, is concentrated by the stomach.(60) A year later Lipschutz confirmed this report and extended the studies to saliva. He found concentration ratios between iodide in gastric juice and in plasma of up to 15, and salivary iodide concentrations up to 7 times the plasma level.(61) Some 12 years later these studies were taken up again by Flexner and his co-workers, who concluded that iodide "is selectively concentrated and secreted by the salivary and gastric glands" of man.(62) Davenport in 1943 tried unsuccessfully to elucidate the mechanism of iodide concentration by the gastric mucosa of dogs. However, he could show that the gastric excretion of iodide does not involve a diffusion mechanism, such as governs the excretion of chloride, bromide, or the sulfonamides, that the iodide level in the gastric contents is independent of the rate of secretion and the pH of the gastric juice, and that all the iodine in the gastric contents is present as the iodide ion.(63)

Whatever its theoretical basis, the fact that iodide is concentrated by the human stomach was thus well established. It seemed of interest to me to evaluate the

possible use of the gastric excretion of iodide as a function test for the physiology of the stomach. The determination of iodide in low concentrations in biological specimens by the usual chemical methods is notoriously cumbersome. But radioiodide can be determined readily by means of Geiger-Mueller counters. Therefore, I decided to undertake some preliminary studies on the gastric excretion of radioiodide. My aims were

a) to investigate if gastric secretion of radioiodide might be used as a diagnostic tool; and

b) if selective gastric concentrations of radioiodide could be obtained which would be high enough to make the substance feasible for radiation therapy of the stomach.

I studied the gastric excretion of radioiodide on 3 patients, and its salivary excretion on myself. All the subjects were given Lugol's solution prior to the administration of radioiodide to cut down the uptake of radioiodine by the thyroid. The data for these experiments are given in Tables XII to XV.*

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*The radioactivity measurements reported in tables XII to XVI were carried out on the Geiger-Mueller counter of the Children's Hospital. I am indebted to Dr. S. Rapoport for the use of this instrument.

8.2) RESULTS AND DISCUSSION

From the data in Tables XII to XV it is apparent that radioiodide is concentrated heavily in the normal and diseased human stomach. The concentration ratios in the normal individual were definitely higher than the corresponding values in the patients with gastric malignancies. However, the data are insufficient to draw any conclusions concerning the possible value of radioiodide for the diagnosis of gastric disorders.

Radioiodide was also found to be concentrated by the salivary glands, but the concentration ratios were lower than those for the gastric contents (Table XVI).

One experiment on a dog, in which the distribution of radioactivity in the gastric wall was assayed, indicated that radioiodide is concentrated largely by the pyloric portion of the stomach.

In view of these findings it appears to be worth while to investigate the gastric excretion of radioiodide in health and disease more thoroughly. It may prove of value in diagnosis, particularly for the evaluation of pyloric secretion for which no test has been developed thus far. Also, it appears likely that administration of radioiodide may result in sufficiently high and localized radiation to the stomach to be of use therapeutically. This may hold true not only in malignant diseases, but also in benign

Table XII

J. B. No. 190138
Extensive carcinoma of the stomach with pyloric obstruction.

Received 120 min. Lugol's sol. over 4 day period, then 2,000 microc. I^{131} with 100 mg. NaI in 20 cc. saline i.v. (2-4-44)

Samples	Time after inj. of NaI	Vol. cc.	pH	CMG corrected for 2-15-44	Concentration Ratios
Gastric juice	0	21	4	0	-
" "	15'	19	7	430	3.7
" "	30'	16	7	980	13
" "	45'	22	7	1510	22
" "	60'	14	7.5	1310	22
" "	90'	23	7.5	1370	23
" "	120'	23	4	2100	35
Plasma	10'	-	-	117	
"	30'	-	-	75	
"	60'	-	-	60	
"	24 hrs.	-	-	32	
"	72 hrs.	-	-	11	
"	5 days	-	-	5	
"	8 days	-	-	2	
Urine	0-24 hrs.	1400	-	560	

Table XIII

J. B. No. 190138

Received 70 min. Lugol's solution over 3 day period, then 1,000 microc. I^{131} with 100 mg. NaI in 30 cc saline i.v. (2-4-44)

Samples	Time after inj. of NaI	Vol. cc.	pH	CMG corrected for 3-24-44	Concentration Ratios
Gastric juice	0	33	6.8	0	-
" "	15'	65	6.8	653	19
" "	30'	22	6.4	680	20
" "	45'	28	7.5	460	14
" "	60'	18	7.8	395	15
" "	75'	12	7.4	329	12
" "	90'	8	7.5	263	10
" "	120'	38	7.5	665	32
Plasma	30'	-	-	34	
"	120'	-	-	21	
"	29 hrs.	-	-	5	
Urine	0-24 hrs.	650	-	780	
"	24-48 hrs.	1150	-	86	
"	48-72 hrs.	2500	-	7	

Table XIV

U.G.P.

Linnitis Plastica

Received 100 mg. NaI in saline i.v. (3-21-44)
then 250 microc. I^{131} with 100 mg. NaI in 15 cc saline i.v. (3-22-44)

Samples	Time after inj. of NaI	Vol. cc.	pH	CMG corrected for 3-24-44	Concentration Ratios
Gastric juice	0	33	6.8	0	-
" "	15'	65	6.8	653	19
" "	30'	22	6.4	680	20
" "	45'	28	7.5	460	14
" "	60'	18	7.8	395	15
" "	75'	12	7.4	329	12
" "	90'	8	7.5	263	10
" "	120'	38	7.5	665	32
Plasma	30'	-	-	34	
"	120'	-	-	21	
"	29 hrs.	-	-	5	
Urine	0-24 hrs.	650	-	780	
"	24-48 hrs.	1150	-	86	
"	48-72 hrs.	2500	-	7	

Table XV

D.J.

Normal Stomach

Received 250 microc. I^{131} with 100 mg. NaI in 15 cc saline i.v. (3-21-44)

Samples	Time after inj. of NaI	Vol. cc.	pH	CMG corrected for 3-29-44	Concentration Ratios
Gastric juice	0	27	2.3	0	-
" "	15'	24	1.7	480	11
" "	30'	21	1.8	1750	40
" "	45'	17	1.8	1650	41
" "	60'	16	1.8	1600	43
" "	90'	31	1.8	1220	41
" "	120'	34	1.8	920	38
" "	150'	34	1.7	720	40
" "	180'	42	1.8	570	48
" " *	2 days	50	4.8	8	-
Plasma	30'	-	-	44	
"	120'	-	-	24	
"	2 days	-	-	0	

* 4+ Bile

Table XVI

Excretion of Radioiodide in Human Saliva

Subject: H.S.B. chewed gum

Received 300 microc. I^{131} with 100 mg NaI in 20 cc saline i.v.

Samples	Time after inj. of NaI	Vol. cc.	CMG Corrected for 5- 5-44	Concentration Ratios
Basal	0	33	0	-
Saliva	15'	24	1000	4.2
"	30'	39	540	2.7
"	45'	29	550	3.5
"	60'	47	350	3.1
"	90'	81	310	12
"	120'	91	290	13
"	150'	89	280	15
"	180'	68	280	16
Plasma	10'	-	240	-
"	90'	-	25	-
"	180'	-	17	-

peptic ulcers, it is known that radiation therapy of the stomach can lead to complete histamine achlorhydria.(64) In the management of the peptic ulcer patient reduction of gastric secretion and acidity is of paramount importance, and radioiodide may become the answer to this problem.

9) SUMMARY

The development of oncology and recent advances in this science are reviewed briefly from the standpoint of a chemist. Attention is then focussed on carcinoma of the stomach in man. Studies on the gastric excretion of dyes by other workers led the author to investigations on the secretion of sulfonamides by the human stomach, with the aim of developing a test for the early diagnosis of gastric cancer. Three sulfonamides (sulfathiazole, sulfadiazine, and sulfapyridine) were studied. None of them were found to be applicable to the early detection of gastric malignancies. The strikingly high sulfapyridine levels in the gastric contests of patients with normal gastric acidity stimulated a theoretical investigation concerning the mechanism of the gastric secretion of sulfonamide drugs. It could be shown that the distribution of these compounds between gastric juice and plasma is largely conditioned by their K_a and K_b, and the difference in the pH of gastric juice and plasma. An equation correlating these quantities with the concentration ratio of the drugs (conc. of drug in gastric juice/ conc. of drug in plasma) was developed. It seems reasonable to expect that this equation will not only hold for the distribution of sulfonamides between the blood and the gastric contents, but also for organs other than the stomach, and for acidic or basic organic compounds of low to medium

molecular weight in general. It may thus become useful in predicting the distribution of medicinals in the body; particularly, it may help in predicting the localization of radioactive organic compounds related to sulfonamides physicochemically.

The possible use of radioactive organic compounds which localize selectively in certain tissues and organs in the diagnosis and treatment of malignant neoplastic diseases is discussed. It is pointed out that a variety of radioactive organic compounds which may show selective localization in the body can be obtained by incorporating radioiodine (I^{131}) in organic molecules. Particular attention is paid to derivatives of azo-dyes and sulfonamides. Compounds of this type were prepared by iodination with elementary iodine in alkaline medium, with iodine monochloride in acid medium, and by the Sandmeyer reaction. They served as models for radioiodo derivatives.

The synthesis of radioiodo derivatives from sodium radioiodide is described. Radioiodosulfanilamide and radioiodosulfapyridine were obtained in excellent yields with respect to radioiodine by treating an acid aqueous solution of the parent sulfonamide, containing sodium radioiodide of the desired activity, with iodine monochloride. Radioiososulfonamides can be diazotized and coupled. They may thus be used to prepare a great variety of radioiodo compounds. Radioiodo-Trypan Blue was obtained from Trypan Blue and

sodium radioiodide by the Sandmeyer reaction.

Radioiodo-Trypan Blue was singled out for some preliminary physiological studies. It was found suitable for further clinical investigation.

Since iodide itself is concentrated by the stomach with a considerable degree of selectivity, some preliminary studies on the secretion of radioiodide by the normal and diseased human stomach were carried out. Following the intravenous injection of sodium radioiodide the gastric juice was found to contain up to 48 times the activity present in the plasma. The possible use of radioiodide as a diagnostic tool and as a means of administering radiotherapy is outlined.

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