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Studies on Iontophoresis

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STUDIES ON IONTOPHORESIS

by

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LIFE

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INTRODUCTION

The fundamental facts of ionization and electrolysis are well known. An acid, base, or salt when dissolved in water becomes dissociated into positively charged ions, called cations, and negatively charges ions, called anions. Such a solution will conduct an electric current. If we introduce into it the positive pole (anode) and the negative pole (cathode) from a voltage source, a flow of current between these poles occurs by nature of the potential and mobility of the ionized substances.

Thus an electropositive ion in solution is attracted to the negative pole (cathode) and the electronegative ion migrates to the positive pole (anode). This follows the fundamental law of electrical charges that like charges repel each other and unlike charges attract each other. In this way positively charged ions will accumulate in the region of the cathode and negatively charged ions will accumulate in the region of the anode.

A living organism, among other things, is essentially a solution of many chemicals which dissociate ionically in the aqueous media in which they are dissolved. It is therefore a good conductor of electricity. If one electrode from a voltage source is applied to a point on the body and the second electrode applied to another part removed from the first, a current may be passed through the circuit and the appropriate ions will accumulate at the

appropriate poles. If a fairly large concentration of a positively charged drug in aqueous solution is available at the anode of such a circuit, in this case the "active electrode", a certain number of such positive ions and therefore a certain quantity of the drug, must be transported to the opposite or negative pole. Such a process of application or introduction of a chemical or drug substances into living tissues by using an electrical current, has been termed iontophoresis.

Electrophoresis, by definition, is a movement of a colloid (dispersed phase) in the presence of an electrical field, with the dispersion medium held constant. For purposes of this discussion electrophoresis should not be used to describe the process of introducing chemicals into living tissues since it is generally defined as a movement of colloidal substances while we are concerned with soluable ionisable materials. Similarly, electroosmosis as a term should not be used since it is defined as a movement of a liquid (dispersion medium) while the dispersed phase is held constant. In contradistinction to some of the literature which has used these three terms interchangeably, we wish to emphasize that in our opinion iontophoresis should be the only term applied to this general process. Some degree of either or both electrophoresis and electroosmosis may take place when chemical agents are applied to the body with the aid of an electrical circuit, but generally we apply ions in solution, not colloids.

The introduction of drugs into the human body by means of an electrical current has fascinated medical physicists for more than a century. According to Turrell (1921), such a form of medication was described as long ago as 1747 by Veratti. Jones (1907) stated that Palaprat claimed to have introduced

iodine into the tissues in this way in 1833. Frankenhauser (1906) in 1898 was very successful in introducing many drugs into the body by the use of galvanic current. The general application of the theory, as a practical exercise in therapeutics, must be credited to Leduc (1907), who, in a series of papers beginning in 1900, brought it prominently before the medical profession.

Within the past twenty years there have developed, in general, three schools of thought as to events taking place during iontophoresis.

(1) Iontophoresis produces a purely local effect. Its penetration is not deep enough to induce significant effects systemically.

(2) The substance used in iontophoresis moves in a "tissue plane". Its movement through the body of the subject is due to the electrical field set up between the two electrodes, and it follows this electrical plane more or less irrespective of biological media.

(3) Iontophoresis produces a systemic or remote effect. This involves the penetration of the substance into the blood stream and its subsequent distribution to all parts of the body.

We will review the available evidence for each of these theories, even though it is apparent that many of the articles relating to them do not present well controlled scientific experiments.

IONTOPHORESIS - LOCAL EFFECT

Jurgens (1932) showed in his experiments that trivalent iron (ferric chloride, ferric sulfate and ferric carbonate) readily passes through the skin of the mouse under the influence of iontophoresis and was deposited in the subcutaneous depots. This was demonstrated by local tissue staining technique. No general body tissue studies were made.

Bredall (1939) attempted iontophoresis of copper with human patients afflicted with cervicitis. He found that this procedure resulted in lining the vaginal part of the cervix with copper crystals. It produced a local action and presented an improved treatment for this disease. Haggard (1939) treated thirty-seven human patients using iontophoresis with copper sulfate, locally, for fungus infections of the feet. He obtained thirty-six recoveries. With seventy-eight human patients afflicted with epidermophyton infection, Jersild (1939) performed local copper iontophoresis and obtained seventy-six cures. Gunderson (1940) claimed success in treating fungus infections with copper sulfate using iontophoresis. Out of six patients, he described five recoveries. Greenwood et al (1941) later reported that iontophoresis with copper sulfate was not the factor necessary for the clearing up of the infection, but that the heat itself was responsible. Solomon (1942) confirmed earlier work that iontophoresis with copper sulfate was very useful in treating dermatophytosis. He cleared up the infection in thirty-three out of forty human patients using local ion transfer therapy. He contended that the copper ions were deposited locally in the skin to a depth sufficient to reach the fungus situated chiefly in the horny layers of the epidermis. The action of the current deposited the

free fungicidal copper ions in the skin, which in turn produced a protein precipitation. Lahey and Byrnes (1946) concurred with the success of the work of early experimentors in regard to the use of copper sulfate by iontophoresis for fungus infections.

Harpuder (1937) maintained that penetration in human skin did not go further than through the rete malpighii and in the region of the hair follicles and coil glands as shown with methylene blue dye. He considered this an intradermal form of therapy. The author carried out his studies on a number of human patients. Roskin et al (1940) produced vital staining with iontophoresis. He found that penetration of methylene blue dye into the muscles of the mouse required several successive days of treatment. Molitor (1943) held that drug administration by ion transfer produced long lasting local effects with a minimum of systemic effects, provided the drug used was unstable in the body or was rapidly excreted. Samailov (1945) observed that experiments with iontophoresis of calcium into the eyes of rabbits indicated the probability of direct entry of calcium ions into the eyeball. The amount of calcium ions thus entering could not be detected chemically. However the retinal tissue gave a characteristic contractile reaction which is found with increased calcium concentration. Bernshtein (1946) in his experiments with iontophoresis, on specific sensitivity of eyes of animals infected with tuberculosis, found desensitization of the affected eye tissues after iontophoresis with calcium. The work of Erlanger (1939) showed that iontophoretic medications in general were valuable in treatment of various pathological conditions of the anterior parts of the eye. Thau et al (1940) observed, with work on four human patients, that application of sodium chloride by either the positive or negative galvanic current around the

eye caused a general parasympathetic effect, slight narrowing of the palpebral fissure, decreased size of the pupil and a persistent lowering of the intraocular tension. Clark et al (1942) showed in extensive work with twenty-four rats that penetration of sulfonamides through the intact skin was better when used with electrical current than with any other means of local application. Boyd (1942) observed that iontophoresis with sodium sulfathiazole in rabbits produced a threefold increase in the sulfathiazole concentration of the cornea and the aqueous humor when compared with the use of a simple corneal bath of sodium sulfathiazole of equal duration. The final concentration of sulfathiazole was measured by bioassay of the aqueous humor. Von Sallmann (1943) was very successful in introducing atropine and scopolamine into the eyes of thirty-four rabbits. The concentration entering the eye was determined by the pharmacological effect on the eye. Von Sallmann et al (1944) found that iontophoresis of penicillin into the eyes of rabbits increased the concentration of penicillin in the aqueous humor by ten times as much as the corneal bath of penicillin without electrical current. Continuing with this work, Von Sallmann (1944) observed that approximately the same concentration of penicillin and sulfacetamide are found in the aqueous humor of rabbits after the iontophoretic application of a solution containing both their sodium salts as after the iontophoretic application of a solution containing the separate respective salt. Culliman (1944) found that iontophoresis with sulfathiazole offered a safe method for the treatment of staphylococcus infections of the skin. It produced an increase in the local blood supply. Hamilton and Patterson (1946) disputed the therapeutic effect of penicillin used in iontophoresis. They contended

that it had been impossible, in the conditions described, to demonstrate any movement of penicillin under the influence of the electric current. Work done by Popkin (1946) agreed with the reports by Hamilton and Patterson that penicillin was not ionized and therefore would not pass through the skin. Bellows et al (1950) found in their work on twelve rabbits that aureomycin showed very little penetration into the aqueous humor after local iontophoresis. However, Grainwell (1949) used iontophoresis with streptomycin for clinical ophthalmology with great success. He treated four patients with uveitis and obtained improvement in all four. Smith (1951) in a review states that the use of iontophoresis for the introduction of certain drugs into the eye was based upon sound scientific principles.

Montgomery et al (1938) concluded that acetyl-beta-methyl choline chloride administered by iontophoresis increased the rate of peripheral blood flow but it was only on a local basis. He reported these results on three patients. Abramson et al (1942) ran experiments on patients using mecholyl by iontophoresis. They noted an increase of blood flow even for some time after the experiment was terminated due to vasodilation of cutaneous blood vessels. Controls were run with this experiment. Smyth and Freyberg (1942) treated rhumatoid arthritis with vasodilating drugs by local iontophoresis in twenty-eight human patients. Seventy-nine per cent of the patients were partially relieved. Localized temporary increases in circulation were produced. Saylor et al (1936) treated chronic varicose ulcers by iontophoresis with acetyl- beta-methyl choline and obtained twenty-five recoveries out of twenty-six patients. Schmidt (1946) used histamine ion transfer over spastic muscles with great

success in relief of local spasm. Abramson and Grosberg (1949) made a comparison of the anti histamine action of pyribenzamine and epinephrine introduced into human skin by iontophoresis. They concluded that epinephrine was one of the most powerful anti histamine drugs, one thousand times more effective than pyribenzamine.

Gordon and Darling (1949) noted that threshold values to electrical stimulation of a suitable nerve muscle preparation were not altered after local iontophoresis of the muscle with d- tubocurarine. Observations were made on six rabbits and controls were run. Neuwirth (1949) claimed as a result of his experiments that curare extracts were moved into the tissue of human patients by galvanic current. He stated that the skin of the iontophoresis area showed vasodilation. This was contrary to the findings of Gordon and Darling as they indicated there was no penetration of d- tubocurarine into the skin of rabbits.

In iontophoretic studies of skin of five human patients using extracts of various grasses, Shilkret (1942) showed that the skin reaction following iontophoresis is parallel to that produced by intracutaneous injection.

Okunev (1935) in his investigations found when hydrogen ions were applied by iontophoresis to experimental mice tumors placed between two electrodes, the rate of growth of tumor tissue was retarded. Microscopic examination of tissues and the changes resulting therein indicated that the hydrogen ions had penetrated a depth of 1.5 centimeters in these experiments.

Barakin (1939) found benefit in treating cases of neuralgia by local iontophoresis with aconitin in human patients. He found an analgesic action with an improvement in the circulation. Dancik and Degrott (1951) used gal-

vanic current with saline applied to the positive pole over ecchymotic areas near the optic orbit of patients. Experiments were done on forty human patients and forty very rapid recoveries were reported. They concluded that locally the positive pole with saline causes constriction of blood vessels while the negative pole causes dilation.

IONTOPHORESIS - "TISSUE PLANE" EFFECT

Abramson et al (1937) observed after a series of experiments on human patients that electrolytes seemed to hinder the electrical migration of histamine. He attributed this to the lowering of the electro - kinetic potential of the skin. Abramson (1938) stated that histamine in an electrical field moved into the skin due to a charge. He called this process electrophoresis. He contended after experiments with patients using iontophoresis with ragweed extract that the positively charged water molecules set up an electro - osmotic stream through the pores of the skin. He found that histamine was transported into the skin by application of the negative electrode. However, he was unable to remove it on application of the negative pole to the same area. Abramson (1939) successfully introduced ragweed extract into the body of human patients. He permitted different quantities to be administered by varying three parameters: a) electrode area, b) current and c) concentration of the solution. Later (1941) he described his electrophoretic theory of the transfer of ragweed pollen. It was mainly the "tissue plane" hypothesis. He also tried administration of histamine and adrenalin into the skin of human patients. He attempted to explain these results in terms of his electrophoretic theory. Abramson in his later publications apparently looked on the mechanism of iontophoresis as producing a systemic effect rather than being solely a local or electrophoretic phenomenon.

Walzer and Golan (1945), following the Abramson hypothesis, reported the transportation of antigen by iontophoresis through the bodies of a chain of three human subjects in the same circuit. The results were considered positive

by the production of a characteristic wheal at the sensitized distal site. Walzer and Golan (1946) performed further experiments in this vein one year later. In this case they reported that the antigen had been transported through a chain of five human subjects by iontophoresis. The used five milliamperes of current for fifteen to thirty minutes. This transport was indicated by the formation of a wheal in a previously sensitized site, on the subject who was most distal from the area of local antigen- electrode application. The authors contended that the antigen was not present in the circulation but moved directly in a charged state along the path of the electric current between the two poles.

IONTOPHORESIS - SYSTEMIC EFFECT

Kotkis and Melchionna (1935) used iontophoresis of acetyl beta methyl choline chloride in eighteen dogs. They noted variable effects on the heart rate, a fall in blood pressure, increased depth of respiration, marked salivation, bloody defecation and forceful urination. This was definitely a systemic effect. Saline controls were used. Jacoby (1936) observed generalized vasodilation, sweating, marked flushing, marked salivation and blood pressure fall in treatment of pelvic inflammation by iontophoresis with acetyl beta methyl choline chloride in human patients. Martin et al (1937) used iontophoresis with mecholyl in treating arthritis with thirty-six patients having infectious arthritis and forty-seven having metabolic arthritis. Relief was obtained in 79 per cent of the metabolic form and 57 per cent of the infectious type. Blood pressure fall, increase in pulse rate, increase in persistalsis, diuresis and prolonged local sweating were noted during the experiments. Controls were run using saline. Loman et al (1937) found that the administration of Mecholyl by iontophoresis produced an alkaline mucous gastric juice as effectively as it did when given by subcutaneous injection. The former treatment had the advantages of not causing so sudden a drop in blood pressure, of having less explosive effects and of markedly increasing the period of time of secretory effects. Administration of prostigmine before iontophoresis of mecholyl enhanced the effects of the latter. Work of this nature was done on ten human subjects. Shortly afterwards Loman et al (1939) found in ten patients that hypertension produced by benzedrine sulfate was markedly reduced by iontophoresis with mecholyl. In three out of eight cases of senile hypertension the administra-

tion of prostigmine prior to iontophoresis with mecholyl reduced the blood pressure to normal. Montgomery et al (1938) found that iontophoresis in the dog with acetyl beta methyl choline chloride produced predominantly systemic rather than local effects. It is noteworthy to mention that in similar experiments by this group with human beings local effects prevailed as was mentioned in the previous section. Macht et al (1948) treated six patients with iontophoresis using acetyl beta methyl choline chloride. The iontophoresis resulted in a significant increase in blood flow of the hand manifested by flushing and sweating of the hand. They also used controls with saline where entirely negative results were obtained. Abramson (1949) continued his work on histamine iontophoresis with human patients suffering from multiple sclerosis reporting very successful therapy by this method.

Frey et al (1941) likewise held that the symptoms of multiple sclerosis were lessened by various iontophoretically administered vasodilating substances. Kling (1940) showed that histamine administered by iontophoresis to patients produced local vasodilation while administration of doryl showed a greater preponderance of systemic reactions.

Inoue et al (1938) noted that in therapeutic application of hydrofluoric acid by iontophoresis in Basedows disease and hypertonia the blood pressure and the subjective symptoms were usually improved. Continuing with this work, Inoue et al (1939) observed that iontophoresis with hydrofluoric acid decreased the iodide and increased the fluoride in the blood. Calcium was slightly reduced, sodium and potassium were increased, magnesium was unaltered and blood sugar was decreased. Simizu (1941) used iontophoresis of a hydrofluoric

acid solution on normal rabbits and found the oxygen requirements decreased significantly with each application. Mukhamedova et al (1938) treated one hundred and forty rabbits for uterine hemorrhage of different etiology by iontophoresis with potassium iodide. With few exceptions the therapeutic results were good. Experiments on rabbits showed the hemostatic result of the iontophoresis to be due to an effect on the ovary increasing the production of lutein. Bourguignon (1944) introduced iodide, bromide, calcium, magnesium, iron, potassium and sodium ions by iontophoresis into the eyes of five humans and one rabbit. The retina was examined with an opthalmoscope. In all cases except with sodium chloride solution or distilled water, there was vasodilation of the retina and increase in peripheral blood pressure as measured in the arm. One year later Bourguignon (1945) observed tha action of calcium by iontophoresis in the rat before and after hypophysectomy. He found in normal rats that passage of current with calcium produced turgescence of the graffian follicles and horns of the uterus. In rats hypophysectomized five to ten days previously no side effects were produced by the calcium iontophoresis. Barnett (1937) showed that in five cases of bronchial asthma treated by transcerebro-spinal iontophoresis with calcium, permanent relief was obtained in three cases exhibiting a functional type of disturbance and temporary relisf in two cases showing chronic perihilar lesions. Dobrokotova (1946) experimented on human skin with equimolar solutions of binary mixtures of adrenalin with codeine, with quinine, with sodium chloride and with calcium chloride. For all of these mixtures except codeine and adrenalin there was a spasm of the vessels, typical of that produced by adrenalin. In the case of codeine and adrenalin there was

no spasm of the vessels showing the predominating action of codeine. He therefore concluded that the predominating action in iontophoresis is due to the greater pharmacological activity of the substances rather than their physical properties under the influence of the current.

Payne (1937) treated human patients for nasal allergic diseases by iontophoresis with galvanic current and the symptoms were relieved promptly. Ickowicz (1937) observed that disodium hydrogen arsenate and nickel acetate produced caryoclasis in the thymus when introduced into the organism by iontophoresis as well as by subcutaneous injection. Garb (1951) observed the effects of various ions on mammalian heart muscle, in vivo after iontophoresis. These experiments produced electrocardiographic changes. Blood calcium concentration was increased by such iontophoresis and simultaneously E. K. G. changes and increased force of contraction of the heart were noted. Controls were run in this set of experiments. Al'bor (1941) noted in patients that zinc iontophoresis prior to the injection of insulin gave a more even and somewhat more gradual drop of blood sugar than insulin alone.

Fiessinger and Gajdos (1935) introduced histamine into the epigastric area of the abdominal wall by iontophoresis and found an improvement in the general circulation of the patients. Levant (1935) used iontophoresis with histamine in ninety arthritic patients. He reported excellent results for the relief of pain and stiffness. Garfin and Pearl (1936) used iontophoresis with histamine for treatment of hay-fever and allied conditions and reported that all forty-six human patients so treated were relieved of their symptoms.

Abramson (1948) administered histamine by iontophoresis to patients

afflicted with multiple sclerosis. He reported considerable success in his endeavor to alleviate the symptoms of this disease. The author noted a decrease of the diastolic and systolic blood pressures, and generalized secondary flushes during the treatment.

We have tried to summarize the available literature dealing with individual substances which have been used in iontophoresis and which have produced a specific effect or effects. This list is found in table I.

TABLE I

LIST OF SUBSTANCES USED IN IONTOPHORESIS

SUBSTANCE	LOCAL EFFECT	REMOTE EFFECT
Acetyl choline	-	Blood pressure effects
Acetyl beta methyl choline chloride	Increased blood flow	-
Aconitine	-	Speeded up circulation
Adrenalin	-	Blood pressure changes
Adrenalin with quinine, NaCl and $CaCl_2$	Spasm of the blood vessels	-
Antigen	Wheal formation	Wheal effect
Atropine	Pupil changes	-
Aureomycin	Microbiological inhibition in local fluids	-
Bromine	-	Vasodilation of the retina and increase of peripheral blood pressure
Calcium	-	Vasodilation of the retina and increase of peripheral blood pressure - also cardiac effects, turgescence and congestion.
Copper	Lines cervix of uterus, tissue penetration	-
Curare	Vasodilation locally	-
Ferrous ion Ferric ions	-	Vasodilation of the retina and increase of peripheral blood pressure

TABLE I (CONTINUED)

LIST OF SUBSTANCES USED IN IONTOPHORESIS

SUBSTANCE	LOCAL EFFECT	REMOTE EFFECT
$FeCO_3$	Histological penetration	-
$FeCl_2$	Histological penetration	-
$FeSO_4$	Histological penetration	-
Histamine	Increased local circulation	General flushes
Histamine & adrenalin	Blanching of the skin	-
Hydrofluoric acid	-	Increased serum Na, K and F ions, decreased serum Ca and I ions and decreased blood sugar
Hydrogen ions	Inhibition of tumor growth	-
Iodine		Vasodilation of the retina and increase of peripheral blood pressure
Magnesium ions		Vasodilation of the retina and increase of peripheral blood pressure
Mecholyl	Vasodilation of blood vessels	Alkaline mucus gastric juice secretion
Methylene blue	Staining of skin and muscles	-
Nickel acetate	-	Produced caryeclasis

TABLE I (CONTINUED)

LIST OF SUBSTANCES USED IN IONTOPHORESIS

SUBSTANCE	LOCAL EFFECT	REMOTE EFFECT
Penicillin	Microbiological inhibition in local fluids	-
Penicillin & sulfacetamide	Microbiological inhibition in local fluids	-
Potassium ions	-	Vasodilation of the retina and increase of peripheral blood pressure
Pyribenzamine & epinephrine	Whealing in the skin	-
Ragweed extract	Whealing in the skin	-
Scopolamine	Local pupil changes	-
Sodium ions	-	Vasodilation of the retina and increase of peripheral blood pressure
Na_2HASO_4	-	Produced caryoclasis
Sodium sulfathiazole	Microbiological inhibition	-
Streptomycin	Presence in aqueous humor of eye	-
Strychnine sulfate	-	General convulsions
Thiamine	-	Marked accelerated pulse
Zinc	-	Gradual drop of blood sugar after insulin

There are no extensive reports in the literature where an attempt was made to organize and summarize the available experimental work. Such a summary has been made here. From this survey we have presented the three most common schools of thought concerning the effects produced by iontophoresis.

In addition, many of the experiments which have been summarized, do not report the use of adequate controls. The only experiment listing chemical identification in the tissues, of the substance used in iontophoresis, is that of Inoue et al (1938).

Since there is such a wide divergence of opinion in regard to what happens to drugs applied by iontophoresis and many of the experiments which are described in the literature are poorly controlled, we decided to try to clarify some of the differences which are apparent. For this purpose we undertook the present experimental study of the process of iontophoresis.

CHAPTER II

MATERIALS AND METHODS

Male and female albino rats of the Sprague - Dawley strain were used in this study. The animals were fed Purina Fox Chow supplemented by fresh green vegetables and fresh meat at least once a week. The weight range of the animals used was between one hundred and fifty and three hundred grams.

Pentobarbital anesthesia was used for all experiments. It was prepared by taking five hundred milligrams of pentobarital powder and diluting it to one hundred milliliters with distilled water. The dosage range used was thirty to fifty milligrams of pentobarbital per kilogram, intraperitoneally.

The iontophoresis apparatus consisted of two ninety volt batteries connected in series and with an ammeter and potentiometer for regulation of current flow. A switch and the electrodes completed the circuit. The electrodes consisted of wire terminals bound around a piece of moistened cotton and attached to the body of the rat. For convenience, the "driving electrode"; the electrode where the material to be introduced was placed; was attached to the front left foot of the rat, and the "receiving electrode"; the opposite pole; was attached to the middle of the tail of the animal, approximately twenty centimeters from the point of application. The cotton of the "receiving electrode" was moistened in all cases. The "driving electrode" and the "re-

ceiving electrode" represented an area of approximately six square centimeters. The substance to be applied to the "driving electrode" was measured with a pipette and carefully dropped on to the cotton, making sure none escaped into the adjacent area and that the cotton was saturated. Five milliamperes of current for a duration of one hour was used in all cases, as the period of application of the iontophoresis.

With radioactive tracers the "driving electrode" was prepared in the same manner as outlined. In addition, a piece of glass tubing was slipped over the electrode area and sealed with paraffin at its innermost end. This served to prevent any superficial contamination of the adjacent tissues with the radioactive material. Appropriate aqueous solutions of the radio-isotopes were placed in the open end of the tube of the "driving electrode" by means of a pipette, thus effectively moistening the cotton, which served as electrode contact.

SAMPLES FOR CHEMICAL TESTS AND SPECTROPHOTOMETRIC MEASUREMENT

a) Blood Plasma

The blood of the animals was taken by cardiac puncture before and after the experiment, one milliliter of the blood was collected each time. It was collected in a heparinized syringe and centrifuged. The plasma was drawn off in a pipette.

b) Urine

The urine of the animals was collected by means of a beaker placed under a metabolism cage. Five milliliters were collected in this manner during the course of five hours before and five hours after the experiment.

c) "Receiving Electrode" Sample Preparation

The "receiving electrode" cotton was washed with distilled water. The washings were evaporated to approximately five milliliters.

SAMPLES FOR RADIOACTIVITY MEASUREMENT

The radioactivity of each of the test samples was measured by counting with an unshielded end window of a Geiger Mueller tube-autoscaler. A comparison sample, which represented a suitable aliquot of the original solution, was used as standard. This eliminated correcting for radioactive decay. The radioactivity of all of the samples was measured in the liquid state, employing the method of Friedman and Hume (1950).

After the iontophoresis was concluded, a series of samples were taken from the animal for assay for radioactivity. Specimens for these test samples were taken from abdominal wall, blood, "driving electrode" bone, "driving electrode" muscle, kidney, liver, "receiving electrode" cotton, "receiving electrode" tissue, thigh muscle, thyroid tissue and urine. Each sample was weighed while wet. In the case where P^{32}, Na^{24} and Ca^{45} were used, they were digested with 10N sulfuric acid and gently heated until complete solution was obtained. Each sample was then decolorized with 30 per cent hydrogen peroxide. The samples were made up to ten milliliters volumetrically. A one milliliter aliquot was taken, placed in a porcelain capsule four centimeters in diameter, and radioactivity counts were taken. With I^{131} and labeled diiodofluorescein the samples were treated as described except 30 per cent potassium hydroxide was substituted as the digesting agent in place of sulfuric acid.

DYES USED IN IONTOPHORESIS

a) Methylene Blue Chloride

Five hundred milligrams of methylene blue chloride powder were weighed out, dissolved in distilled water, and brought to a one hundred milliliter volume with distilled water. This gave a final methylene blue chloride solution of five milligrams per milliliter of solution at a pH of 4.5. Three milliliters of this solution or fifteen milligrams of methylene blue were applied to the "driving electrode", the anode, attached to the front left leg. Blood and urine samples were taken before and after the experiment. The plasma of six animals, run under the same conditions, was pooled and examined by the spectrophotometer for presence of the dye. The "receiving electrode" washings were also examined spectrophotometrically for the presence of the dye. The "receiving electrode" area was observed for any presence of the dye. The animals were autopsied after the experiment to note any penetration of the dye.

b) Sodium Eosinate

The same concentration of sodium eosinate was used. Also the same procedure for the preparation of the solution, its application and the collection of samples, was followed as described under methylene blue. The pH in this case was 4.8.

INORGANIC COMPOUNDS USED IN IONTOPHORESIS

a) Potassium Iodide

A 658 milligram sample of potassium iodide was weighed out and dissolved in one hundred milliliters of distilled water. The final solution therefore contained five milligrams of iodide per milliliter of solution and

was at a pH of 7.9. Five milliliters of the solution, or twenty-five milligrams of iodide, were applied to the "driving electrode", the cathode, attached to the front left leg. The remaining procedure was the same as previously outlined. The starch iodine test was used in an endeavor to detect iodine which had been transported by iontophoresis.

This test was performed following the procedure of Feigl (1947). One milliliter of 2N acetic acid was added to one milliliter of the unknown or the standard potassium iodide solution. This was followed by one drop of 0.1N potassium nitrite solution and one milliliter of 0.2 per cent starch solution. In the presence of iodine a blue coloration appears. Sensitivity tests by this method on urine, blood and distilled water samples to which a standard concentration of iodide had been added, indicated that iodine in an amount of five micrograms per milliliter could be detected.

b) Sodium Borate

A 1.2 gram sample of sodium borate was weighed out and dissolved in one hundred milliliters of distilled water. The final solution therefore contained five milligrams of borate per milliliter of solution at a pH of 9.0. Five milliliters of the solution, or twenty-five milligrams of borate, were applied to the "driving electrode", the cathode. The remaining procedure was the same as previously outlined.

The test used for the detection of borate was turmeric paper. A drop of the test solution, acidified with hydrochloric acid, is placed on a strip of turmeric paper (three by seven millimeters) and dried at 100 C. A red - brown fleck, which turns blue to greenish black on treatment with 1 per

cent sodium hydroxide, indicated the presence of borate—Feigl (1947). Sensitivity tests run here indicates a detection of concentration approximating 0.5 micrograms of borate per drop in urine, blood plasma and distilled water.

c) Copper Sulfate

Two grams of copper sulfate were weighed out and dissolved in one hundred milliliters of distilled water. The final solution therefore contained five milligrams per milliliter of copper and was at a pH of 3.7. Five milliliters of the solution, or twenty-five milligrams of copper, was applied to the "driving electrode", the anode. The remaining procedure was the same as previously outlined.

The o-tolidine ammonium thiocyanate test was used for the detection of copper. O-tolidine and ammonium thiocyanate solution (0.1 grams o-tolidine and 0.5 grams ammonium thiocyanate in five milliliters of acetone) produces a blue coloration in the presence of copper ions—Feigl (1947). Sensitivity experiments run with this test indicated that a concentration of one microgram per milliliter could be detected in urine, blood plasma and distilled water.

d) Mercuric Chloride

A 676 milligram sample of mercuric chloride was weighed out and dissolved in one hundred milliliters of distilled water. The final solution therefore contained five milligrams of mercury per milliliter of solution and was at a pH of 3.1. Five milliliters of the solution, or twenty-five milligrams of mercury, were applied to the "driving electrode", the anode. The remaining procedure was the same as previously outlined.

The test used for the detection of the presence of mercury was

stannous chloride and aniline. A drop of the test solution is placed on a spot plate followed by a drop of 30 per cent stannous chloride solution and a drop of 100 per cent aniline. A black to brown color indicates the presence of mercury—Feigl (1947). Sensitivity tests run with this procedure indicated that a concentration of five micrograms of mercury per milliliter of solution in urine, blood plasma and distilled water could be detected.

ORGANIC DRUGS USED IN IONTOPHORESIS

a) Strychnine Sulfate

A three hundred milligram sample of strychnine sulfate was weighed out and dissolved in one hundred milliliters of distilled water. The final solution contained three milligrams of strychnine sulfate per milliliter of solution at a pH of 4.0. Three milliliters of the solution, or nine milligrams, were applied to the "driving electrode", the anode. The remaining procedure was the same as previously outlined.

The appearance of typical generalized strychnine convulsions would indicate rather widespread strychnine penetration. Experimentally, in the adult rat under thirty to fifty milligrams of pentobarbital per kilogram, it required a subcutaneous injection of at least two milligrams of strychnine sulfate to produce convulsions.

b) Picrotoxin

An aqueous solution of three milligrams of picrotoxin per milliliter was taken and acidified slightly with one drop of 10 per cent hydrochloric acid. The pH of the solution was 3.0. Four milliliters of this picrotoxin solution was applied to the "driving electrode", the anode. This represented

a total of twelve milligrams of picrotoxin. The remaining procedure was the same as outlined previously.

Pharmacological response such as convulsions would offer evidence of the penetration of the picrotoxin. Experimentally, in the rat anesthetized with thirty to fifty milligrams of pentobarbital per kilogram, it required a minimum of 2.4 milligrams of picrotoxin to produce typical tonic-clonic convulsive seizures when given by subcutaneous injection.

c) Nicotine

A three milliliter solution of nicotine sulfate at a pH of 5.6, containing ninety milligrams per milliliter, was applied to the "driving electrode", the anode. This represented a total of 270 milligrams of nicotine sulfate. The remaining procedure was the same as outlined previously.

Evidence for the penetration of the drug would be pharmacological response such as convulsions, fibrillary twitching in skeletal muscle and rapid heart rate. Experimentally, in the rat anesthetized with thirty to fifty milligrams of pentobarbital per kilogram, it required a minimum of sixty milligrams of nicotine sulfate by subcutaneous injection to produce these effects.

d) D-Tubocurarine Chloride

Four milliliters of a twenty unit per milliliter solution of d-tubocurarine chloride or a total of eighty units, at a pH of 5.0 were applied to the "driving electrode", the anode. The remaining procedure was the same as previously outlined.

The sciatic nerve of the rat was exposed in the rear left leg and electric shocks were applied to it. Evidence for the penetration of the drug

would be elicited by an increased threshold and abolition of the response of the leg muscle to the sciatic nerve stimulation. Respiratory paralysis would, of course, also indicate general curarizing action. Subcutaneous injection into the rat, under thirty to fifty milligrams of pentobarbital per kilogram, of fifteen units of d-tubocurarine was found to be the smallest amount which would produce depression and finally abolition of the response of the gastrocnemius muscle (to a standard electrical stimulus).

RADIOACTIVE SUBSTANCES USED IN IONTOPHORESIS

Radio-isotopes used were obtained from the Oak Ridge National Laboratory in Oak Ridge, Tennessee. The radioactive isotopes were usually obtained in solution. In all procedures two dilutions of this solution as delivered were made - 0.1 milliliter of the stock solution diluted to one hundred milliliters with distilled water and 1.0 milliliter of the stock solution diluted to one hundred milliliters with distilled water. Radioactivity counts were taken on both of these diluted samples and used as a basis for calculating the per cent uptake of the material by the animal.

a) P^{32}

Approximately thirty microcuries of P^{32} as sodium phosphate in two milliliters of water at a pH of 2.0 were placed on the "driving electrode", the cathode, in the manner already described. The routine period of iontophoresis was then employed, and various samples were collected and counted as described above.

b) I^{131}

Approximately one hundred microcuries of I^{131} in two milliliters

aqueous solution of sodium hydrogen sulfite at a pH of 11.5 were placed on the "driving electrode", the cathode. The procedure used for iontophoresis and handling samples has been described.

c) Na^{24}

Approximately fifty microcuries of Na^{24} in the form of sodium carbonate in two milliliters aqueous solution at a pH of 10.0 were placed on the "driving electrode", the anode. Iontophoresis was carried out in the manner already described.

d) Ca^{45}

Six hundred two microcuries of Ca^{45} in the form of calcium chloride in three milliliters aqueous solution at a pH of 4.0 were placed on the "driving electrode", the anode.

e) Labeled Diiodofluorescein

Approximately one hundred microcuries of labeled diiodofluorescein in the form of the sodium salt in two milliliters aqueous solution at pH 7.9 were placed on the "driving electrode", the cathode.

CHAPTER III

RESULTS

1) Dye Experiments

a) Methylene Blue

Methylene blue was added to urine, blood plasma and distilled water in a concentration of 0.3 micrograms. The spectrophotometric curves obtained from urine and plasma are shown in figure 1 and figure 2. A maximum optical density at 670 mu. was characteristic of all. Figure 1 and figure 2 also show the result of spectrophotometric examination of blood plasma and urine samples before and after iontophoresis with methylene blue. Since only a limited volume of blood plasma and urine could be obtained from each rat; the blood plasma and urine from six rats, treated in the same manner, was pooled to make these curves.

In six experiments fifteen milligrams of methylene blue were applied at the anode and the current was turned on for one hour at a flow of five milliamperes. The urine was found to contain methylene blue as indicated in figure 1. The peak in this curve corresponded to 0.13 micrograms of methylene blue per milliliter of urine.

The pooled blood plasma after iontophoresis did not reveal the presence of any methylene blue, at least not sufficient to be detected by this method-(see figure 2). The receiving electrode area did not show any staining

resembling that of the dye.

On dissection of these rats, the front left leg, location of the "driving electrode", showed penetration of the dye into the subcutaneous tissue and into the bone. The methylene blue staining appeared to be present for a distance of about one centimeter from the point of application.

An experiment was performed where the poles of the electric circuit were reversed. In this case the methylene blue, a positively charged ion, was at the negative pole. In this type of experiment, after iontophoresis, no spectrophotometric evidence of methylene blue was obtained in urine or blood plasma. Autopsy studies did not reveal any evidence of dye penetration of the tissue locally.

b) Eosin

Eosin, added to urine, blood plasma and distilled water in a concentration of 0.3 micrograms per milliliter, gave a maximum optical density reading at 520 millimicrons. A distinct peak with urine was obtained at a concentration of 0.3 micrograms of eosin per milliliter in urine. In blood 0.3 micrograms per milliliter of eosin was the smallest concentration to give a distinct peak. This is indicated in figure 3 and figure 4. As in the previous case after iontophoresis with methylene blue, urine and blood plasma samples were pooled.

In six experiments, fifteen milligrams of sodium eosinate were applied to the cathode and the current was turned on for one hour at five milliamperes. No eosin was detected in the pooled blood plasma or urine samples. In addition, no eosin stain in the region of this electrode was seen. On dis-

section of these rats, the front left leg, location of the "driving electrode" showed faint and superficial staining, in no case as extensive as that obtained with methylene blue. This staining appeared to be present for a distance of 0.5 centimeter.

Autopsy studies after an experiment with the poles reversed did not reveal any evidence of dye penetration of the tissue locally.

Figure 3 and figure 4 shows the result of spectrophotometric examination of blood plasma and urine samples before and after iontophoresis with eosin.

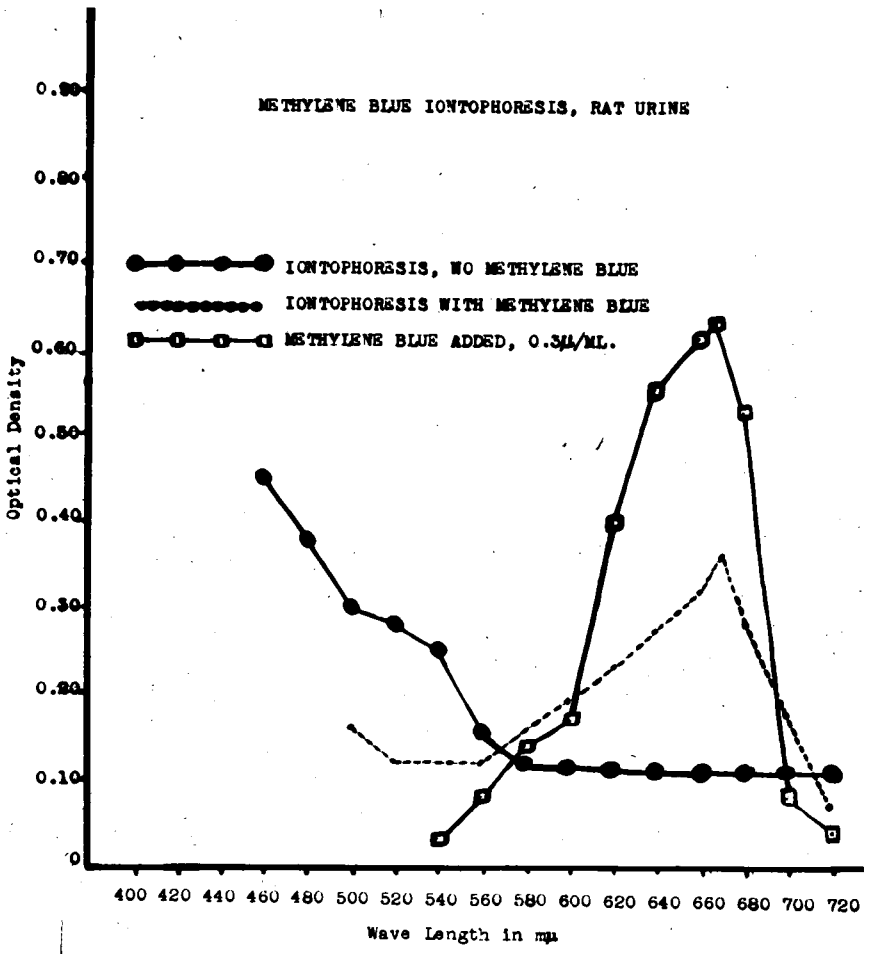

METHYLENE BLUE IONTOPHORESIS, RAT URINE
IONTOPHORESIS, NO METHYLENE BLUE
IONTOPHORESIS WITH METHYLENE BLUE
METHYLENE BLUE ADDED, 0.3µ/ML.
Optical Density
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720
Wave Length in mµ

FIGURE 1

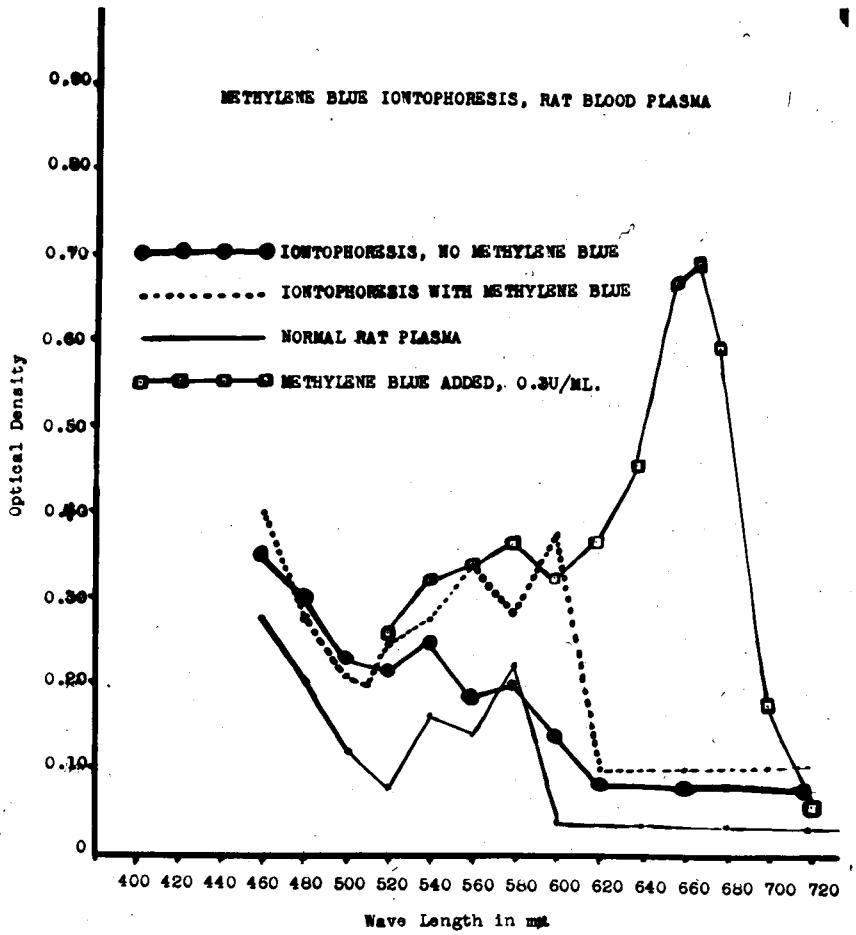

METHYLENE BLUE IONTOPHORESIS, RAT BLOOD PLASMA
IONTOPHORESIS, NO METHYLENE BLUE
IONTOPHORESIS WITH METHYLENE BLUE
NORMAL RAT PLASMA
METHYLENE BLUE ADDED, 0.3U/ML.
Optical Density
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720
Wave Length in mμ

FIGURE 2

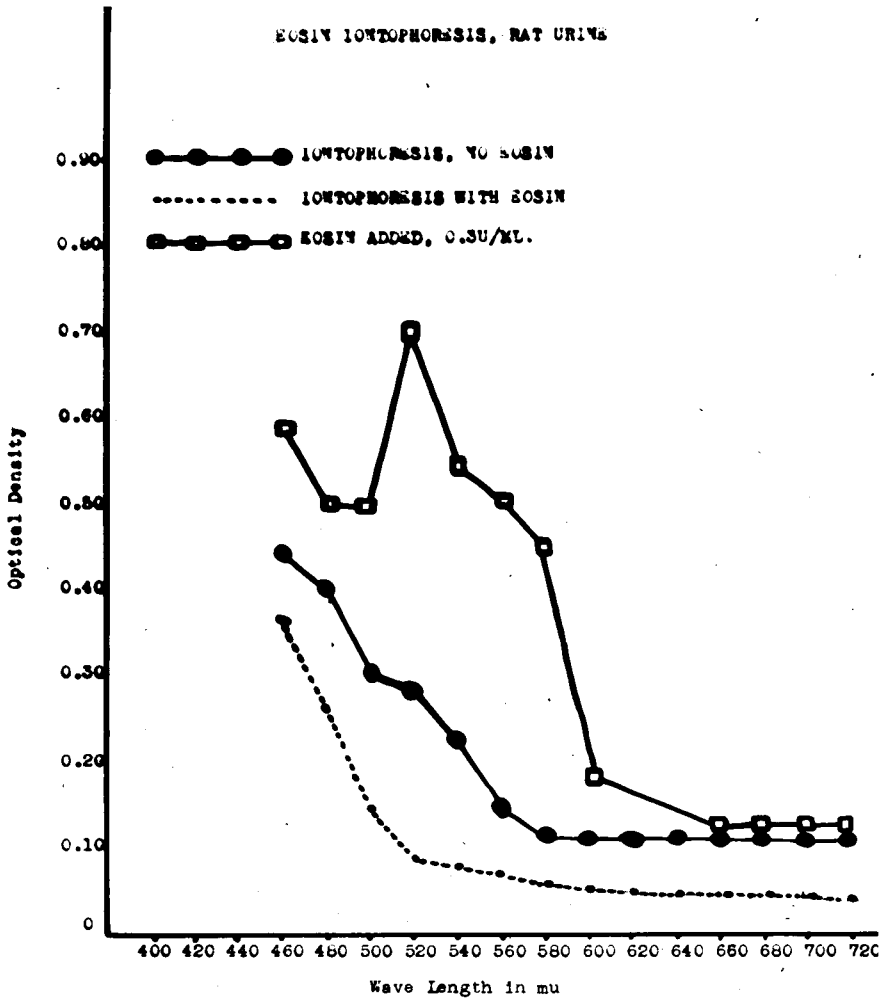

EOSIN IONTOPHORESIS, RAT URINE
IONTOPHORESIS, NO EOSIN
IONTOPHORESIS WITH EOSIN
EOSIN ADDED, 0.3U/ML.
Optical Density
0.90
0.60
0.70
0.50
0.40
0.30
0.20
0.10
0
400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720
Wave Length in mu

FIGURE 3

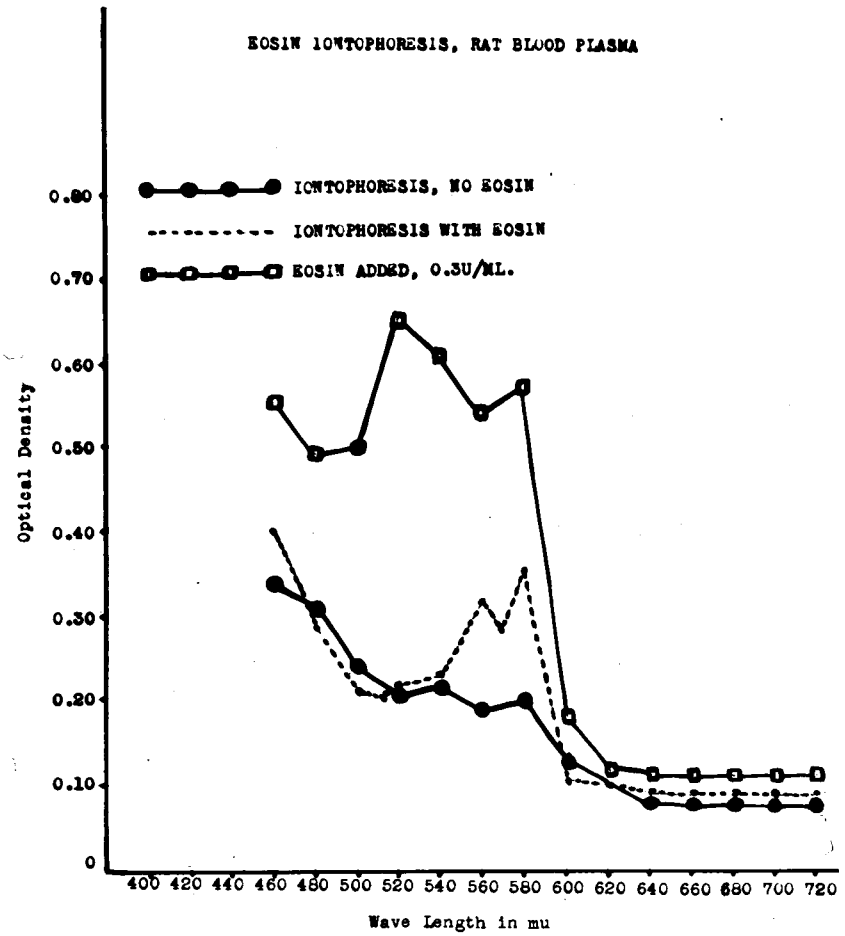

EOSIN IONTOPHORESIS, RAT BLOOD PLASMA
IONTOPHORESIS, NO EOSIN
IONTOPHORESIS WITH EOSIN
EOSIN ADDED, 0.3U/ML.
Optical Density
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720
Wave Length in mu

FIGURE 4

2) Experiments With Inorganic Ions

a) Potassium Iodide

Iontophoresis experiments were carried out with a solution of potassium iodide (twenty-five milligrams of iodide in five milliliters) applied to the cathode at the front left leg. The current was turned on for one hour at five milliamperes. Blood plasma and urine samples were taken immediately before and after the experiment. "Receiving electrode" samples were tested. Three experimental animals and three control animals were used. The poles of the circuit were reversed on three additional animals. The results are represented in table II.

b) Sodium Borate

Sodium borate (twenty-five milligrams of borate in five milliliters) was applied to the "driving electrode", the cathode. Reverse pole experiments were also employed. Results are shown in table III.

c) Copper Sulfate

Iontophoresis experiments were carried out with a solution of copper sulfate (twenty-five milligrams of copper in five milliliters) applied to the "driving electrode" which in this case was the anode. Reversed pole experiments were also employed. The results are shown in table IV.

d) Mercuric Chloride

Mercuric chloride (twenty-five milligrams of mercury in five milliliters) was applied to the "driving electrode", the anode. Reversed pole experiments also were used. Results are shown in table V.

TABLE II

STARCH - IODINE TEST AFTER POTASSIUM IODIDE IONTOPHORESIS

ANIMALS	MATERIALS	URINE BEFORE	URINE AFTER	PLASMA BEFORE	PLASMA AFTER	RECEIVING ELECTRODE AFTER
3	KI (cathode)	-	-	-	-	-
3	KI (anode)	-	-	-	-	-
3	Saline (cathode)	-	-	-	-	-

(-) Indicates negative results.

TABLE III

TURMERIC TESTS AFTER SODIUM BORATE IONTOPHORESIS

ANIMALS	MATERIALS	URINE BEFORE	URINE AFTER	PLASMA BEFORE	PLASMA AFTER	RECEIVING ELECTRODE AFTER
2	$Na_2B_4O_7$ (cathode)	-	-	-	-	-
2	$Na_2B_4O_7$ (anode)	-	-	-	-	-
2	Saline (cathode)	-	-	-	-	-

(-) Indicates negative results.

TABLE IV

O-TOLIDINE - AMMONIUM THIOCYANATE TEST AFTER COPPER IONTOPHORESIS

ANIMALS	MATERIALS	URINE BEFORE	URINE AFTER	PLASMA BEFORE	PLASMA AFTER	RECEIVING ELECTRODE AFTER
4	$CuSO_4$ (anode)	-	-	-	-	-
4	$CuSO_4$ (cathode)	-	-	-	-	-
4	Saline (anode)	-	-	-	-	-

(-) Indicates negative results.

TABLE V

STANNOUS CHLORIDE TEST AFTER MERCURIC CHLORIDE IONTOPHORESIS

ANIMALS	MATERIALS	URINE BEFORE	URINE AFTER	PLASMA BEFORE	PLASMA AFTER	RECEIVING ELECTRODE AFTER
2	$HgCl_2$ (anode)	-	-	-	-	-
2	$HgCl_2$ (cathode)	-	-	-	-	-
2	Saline (anode)	-	-	-	-	-

(-) Indicates negative results.

3) Experiments With Organic Drugs

a) Strychnine Sulfate

Nine milligrams of strychnine sulfate in three milliliters of distilled water were applied to the "driving electrode", the anode. After approximately twenty-five minutes typical strychnine convulsions were seen. Results are shown in table VI.

It was found in rats anesthetized with pentobarbital, that two milligrams of strychnine sulfate in one milliliter of solution by subcutaneous injection produced convulsions similar to those observed in iontophoresis with strychnine.

b) Picrotoxin

Twelve milligrams of picrotoxin in four milliliters of acidified water were applied to the "driving electrode", the anode. Typical picrotoxin seizures resulted within fifty minutes. Results are shown in table VII.

It was found by subcutaneous injection into rats, anesthetized with pentobarbital, that 2.4 milligrams of picrotoxin produced symptoms similar to those observed with the iontophoresis of picrotoxin.

c) Nicotine Sulfate

Two hundred and seventy milligrams of nicotine sulfate in three milliliters of distilled water were applied to the "driving electrode", the anode. Cardiac acceleration, muscular fibrillations and convulsive twitches were seen within forty minutes. Results are shown in table VIII.

It was found by subcutaneous injection that sixty-four milligrams produced responses similar to that observed in iontophoresis.

d) D-Tubocurarine Chloride

Eighty units of d-tubocurarine chloride (twelve milligrams in four milliliters of solution) were applied to the "driving electrode", the anode. One sciatic nerve was exposed and arranged for electrical stimulation with the usual inductorium circuit. Within sixty minutes, the muscle failed to respond to stimuli, respiratory standstill and death occured. Results are shown in table IX.

Subcutaneous injection of fifteen units of d-tubocurarine chloride into an anesthetized rat produced pharmacological response similar to the iontophoresis with eighty units.

Experiments on rabbits, using one hundred units of d-tubocurarine applied to the "driving electrode", the anode, demonstrated the marked increased threshold values for electrical stimulation of a suitable nerve muscle preparation. This is represented in figure 5.

TABLE VI

STRYCHNINE EXPERIMENTS

ANIMALS	SUBSTANCES AND ELECTRODE	RESULTS
4	Strychnine sulfate (anode)	Strychnine convulsions
4	Strychnine sulfate (cathode)	No response
4	Strychnine sulfate (no current)	No response
4	Saline (anode)	No response

TABLE VII

PICROTOXIN EXPERIMENTS

ANIMALS	SUBSTANCE AND ELECTRODE	RESULTS
4	Picrotoxin (anode)	Tonic-clonic convulsions
4	Picrotoxin (cathode)	No response
4	Picrotoxin (no current)	No response
4	Saline (anode)	No response

TABLE VIII

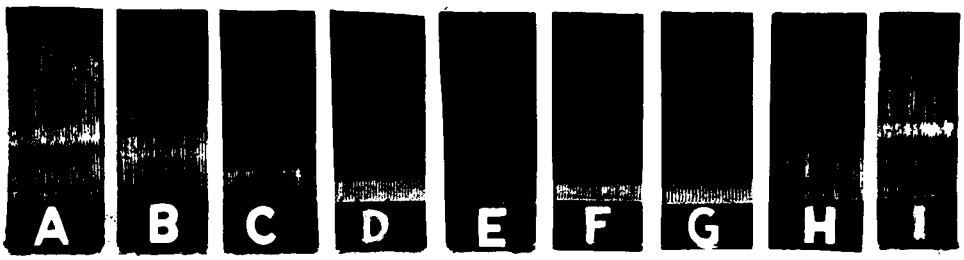
NICOTINE EXPERIMENTS

ANIMALS	SUBSTANCES AND ELECTRODE	RESULTS
4	Nicotine sulfate (anode)	Fibrillary twitchings of the skeletal muscles, slight generalized convulsions and rapid heart rate
4	Nicotine sulfate (cathode)	No response
4	Nicotine sulfate (no current)	No response
4	Saline (anode)	No response

TABLE IX

D-TUBOCURARINE CHLORIDE EXPERIMENTS

ANIMALS	SUBSTANCE AND ELECTRODE	RESULTS
4	D-Tubocurarine chloride	Severe respiratory depression, increased threshold for stimulation of the sciatic nerve and later failure of muscle contraction
4	D-Tubocurarine chloride (cathode)	No response
4	D-Tubocurarine chloride (no current)	No response
4	Saline (anode)	No response



IONTOPHORESIS WITH D- TUBOCURARINE IN RABBIT

Maximum galvanic shocks, 40 per min. to exposed sciatic nerve; gastrocnemius muscle responses recorded.

A- Before iontophoresis. Iontophoresis begun.
B- 15 min.
C- 30 min.
D- 45 min.
E- 60 min. Stop iontophoresis.
F- 75 min.
G- 90 min.
H- 105 min.
I- 120 min.

FIGURE 5

4) Experiments With Radioactive Substances

a) P^{32}

In figure 6 the per cent uptake of the P^{32} by the various tissues and samples is indicated. These results are based on the radioactive count of the P^{32} as found in the various tissues of the rat, as compared to the total counts of the P^{32} which were applied-(Table X). In the case of the P^{32} the initial count was calculated to be 2,048,000 counts per minute for two milliliters of the solution as applied to the electrode, in this case the cathode. Without using any current and applying the P^{32} to the "driving electrode", insignificant quantities were found in the tissues of the rat.

When reversal of the electrodes was employed - the anode being the "driving electrode", only very small amounts of the P^{32} were detected in the tissues. The low uptake of the P^{32} by the tissues is represented by the darkened portion of the bar graph in figure 6.

b) I^{131}

In figure 7 the per cent uptake of the I^{131} by the various tissues and samples are listed. The results were tabulated in the same manner as with P^{32}-(Table X). With I^{131} the total count was 5,111,400 counts per minute for two milliliters of the solution as applied to the electrode, in this case the cathode. After applying I^{131} to the "driving electrode" without using any current, small quantities were found in the various samples. This is illustrated by the darkened portion of the bar graph in figure 7.

With reversal of the electrodes, the anode being the "driving electrode", only small amounts of I^{131} were detected in the various samples.

c) Na^{24}

In figure 8 the per cent uptake of Na^{24} by the various tissues and samples are listed. The results were tabulated in the same manner as with P^{32} and I^{131}-(Table X). With Na^{24} the total count was 630,150 counts per minute for two milliliters of the solution as applied to the electrode, in this case the anode. After applying Na^{24} to the "driving electrode" without using any current, small quantities were found in the various samples.

With reversal of the electrodes, the cathode being the "driving electrode", only small amounts of Na^{24} were detected in the various samples. This is shown by the darkened portion of the bar graph in figure 8.

d) Ca^{45}

In figure 9 the per cent uptake of Ca^{45} by the various tissues and samples are listed. The results were tabulated in the same manner as with P^{32}, I^{131} and Na^{24}-(Table XI). With Ca^{45} the total count was 768,000 counts per minute for three milliliters of the solution as applied to the electrode, in this case the anode. After applying Ca^{45} to the "driving electrode" without using any current, small quantities were found in the various samples.

With reversal of the electrodes, the cathode being the "driving electrode", only small amounts of Ca^{45} were detected in the various samples.

e) Labeled Diiodofluorescein

In figure 10 the per cent uptake of labeled diiodofluroescein by the various tissues and samples are listed. The results were tabulated in the same manner as with all the previous radioactive substances-(Table XI). With labeled diiodofluorescein the total count was 3,076,000 counts per minute for two

milliliters of the solution as applied to the electrode, in this case the cathode. After applying labeled diiodofluorescein the the "driving electrode" without using any current, small quantities were found in the various samples.

With reversal of the electrodes, the anode being the "driving electrode", only small amounts of labeled diiodofluorescein were detected in the various samples.

TABLE X

TISSUE DISTRIBUTION OF RADIO-ISOTOPES IN RAT
AFTER ONE HOUR IONTOPHORESIS

SAMPLES	I^{131} *(5,111,400 c/MIN.) c/MIN. PER GRAM	% UPTAKE PER GRAM	P^{32} *(2,048,000 c/MIN.) c/MIN. PER GRAM	% UPTAKE PER GRAM	Na^{24} *(630,150 c/MIN.) c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving electrode tissue	56,484	1.3300	39,483	1.9270	1,154	0.1800
Urine	12,388	0.2870	14,946	0.7300	567	0.0820
Driving electrode bone	5,229	0.1210	7,946	0.3890	797	0.1260
Thyroid tissue	10,255	0.2370	-	-	-	-
Blood	2,293	0.0530	271	0.0132	565	0.0897
Liver	816	0.0189	1,127	0.0533	263	0.0417
Kidney	1,172	0.0271	1,189	0.0600	575	0.0833
Between electrode tissue	719	0.0162	123	0.0060	274	0.0434
Indifferent tissue	724	0.0168	77	0.0037	241	0.0381
Receiving electrode tissue	353	0.0086	32	0.0002	118	0.0187
Receiving electrode cotton	316	0.0074	14	0.0007	115	0.0183

* Total counts applied

TABLE XI

TISSUE DISTRIBUTION OF RADIO-ISOTOPES IN RAT
AFTER ONE HOUR IONTOPHORESIS

SAMPLES	Ca^{45} *(768,000 c/MIN.) c/MIN. PER GRAM	% UPTAKE PER GRAM	LABELED DIIODOFLUORESCEIN *(3,076,800 c/MIN.) c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving electrode tissue	10,404	1.3980	2,325	0.0759
Urine	3,869	0.5038	497	0.0162
Driving electrode bone	3,217	0.4188	258	0.0084
Thyroid tissue	43	0.0060	179	0.0058
Blood	278	0.0362	64	0.0021
Liver	211	0.0275	36	0.0011
Kidney	128	0.0167	33	0.0010
Between electrode tissue	37	0.0048	34	0.0010
Indifferent tissue	16	0.0021	25	0.0008
Receiving electrode tissue	12	0.0016	8	0.0002
Receiving electrode cotton	11	0.0014	4	0.0001

* Total counts applied

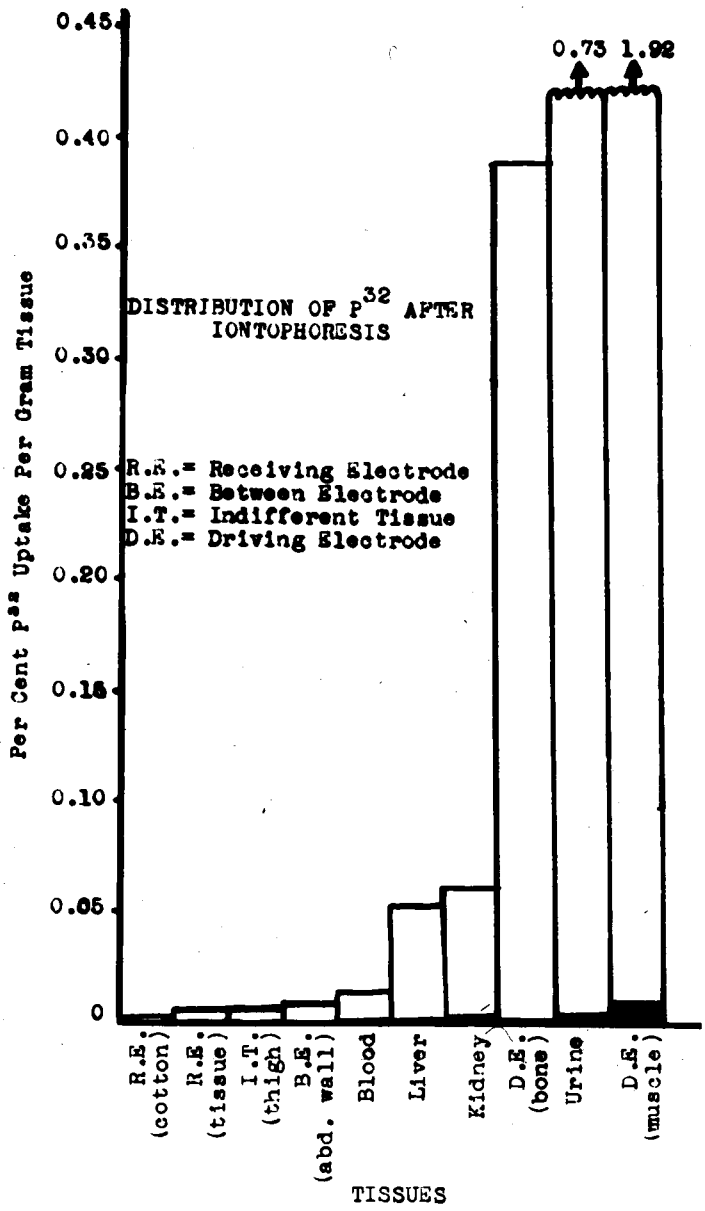

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
0.73
1.92
DISTRIBUTION OF P32 AFTER IONTOPHORESIS
R.E.= Receiving Electrode
B.E.= Between Electrode
I.T.= Indifferent Tissue
D.E.= Driving Electrode
Per Cent P32 Uptake Per Gram Tissue
R.E. (cotton)
R.E. (tissue)
I.T. (thigh)
B.E. (abd. wall)
Blood
Liver
Kidney
D.E. (bone)
Urine
D.E. (muscle)
TISSUES

FIGURE 6

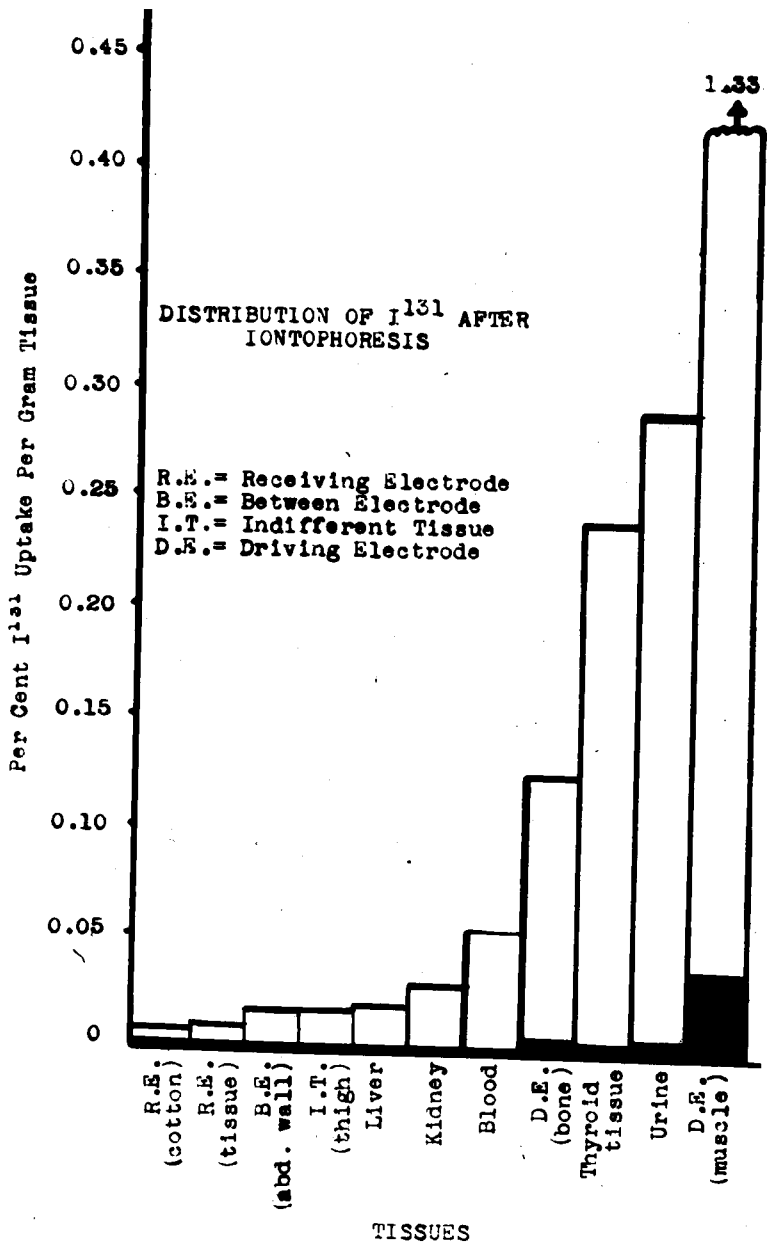

DISTRIBUTION OF I131 AFTER IONTOPHORESIS
R.E.= Receiving Electrode
B.E.= Between Electrode
I.T.= Indifferent Tissue
D.E.= Driving Electrode
Per Cent I131 Uptake Per Gram Tissue
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
1.33
R.E. (cotton)
R.E. (tissue)
B.E. (abd. wall)
I.T. (thigh)
Liver
Kidney
Blood
D.E. (bone)
Thyroid tissue
Urine
D.E. (muscle)
TISSUES

FIGURE 7

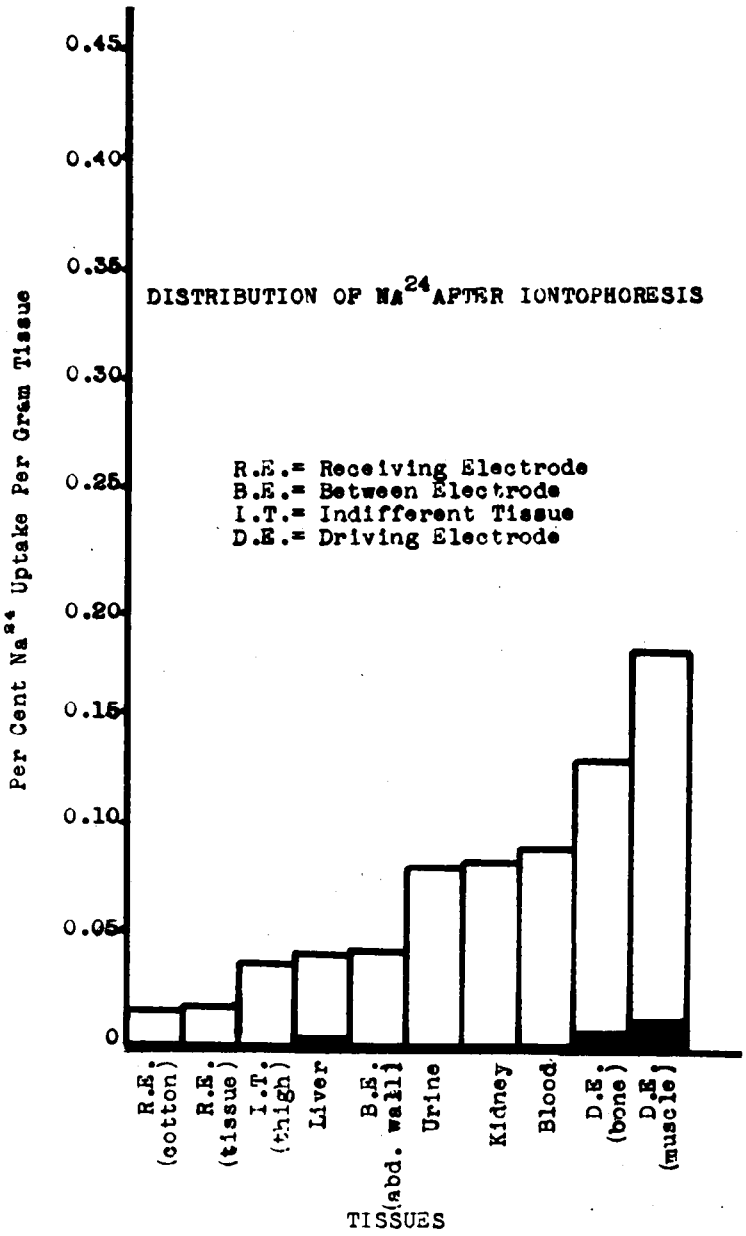

DISTRIBUTION OF Na^{24} AFTER IONTOPHORESIS
R.E.= Receiving Electrode
B.E.= Between Electrode
I.T.= Indifferent Tissue
D.E.= Driving Electrode
Per Cent Na^{24} Uptake Per Gram Tissue
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
R.E. (cotton)
R.E. (tissue)
I.T. (thigh)
Liver
B.E. (abd. wall)
Urine
Kidney
Blood
D.E. (bone)
D.E. (muscle)
TISSUES

FIGURE 8

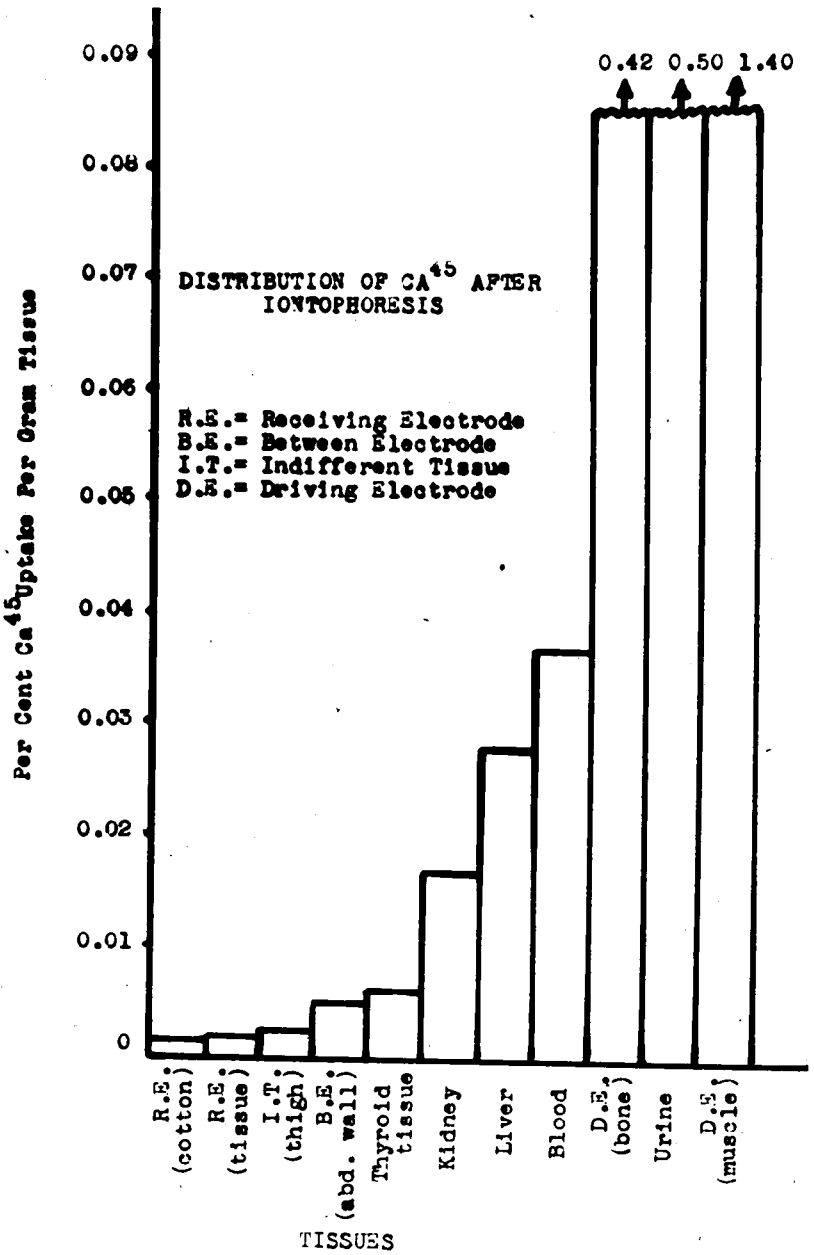

0.42 0.50 1.40
DISTRIBUTION OF CA45 AFTER IONTOPHORESIS
R.E.= Receiving Electrode
B.E.= Between Electrode
I.T.= Indifferent Tissue
D.E.= Driving Electrode
Per Cent Ca45 Uptake Per Gram Tissue
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
R.E. (cotton)
R.E. (tissue)
I.T. (thigh)
B.E. (abd. wall)
Thyroid tissue
Kidney
Liver
Blood
D.E. (bone)
Urine
D.E. (muscle)
TISSUES

FIGURE 9

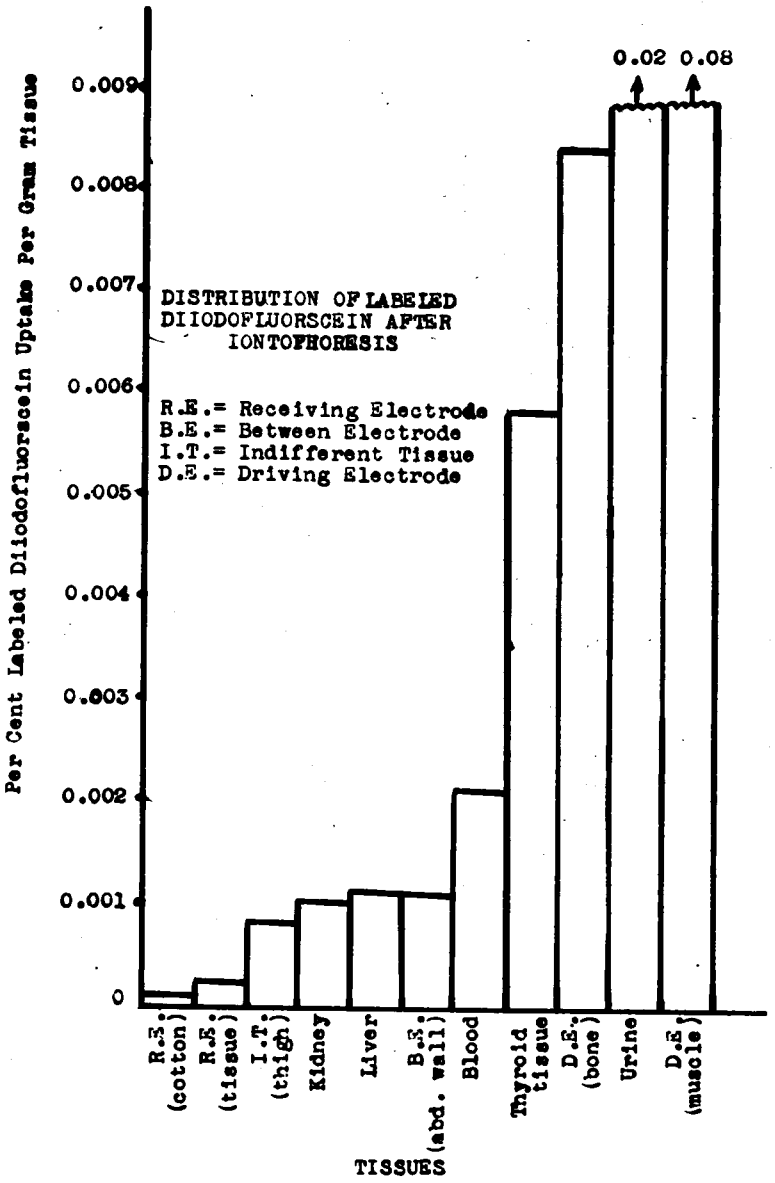

Per Cent Labeled Diiodofluorscein Uptake Per Gram Tissue
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0.02
0.08
DISTRIBUTION OF LABELED DIIODOFLUORSCEIN AFTER IONTOPHORESIS
R.E.= Receiving Electrode
B.E.= Between Electrode
I.T.= Indifferent Tissue
D.E.= Driving Electrode
R.E. (cotton)
R.E. (tissue)
I.T. (thigh)
Kidney
Liver
B.E. (abd. wall)
Blood
Thyroid tissue
D.E. (bone)
Urine
D.E. (muscle)
TISSUES

FIGURE 10

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The experiments with inorganic ions using chemical tests have not demonstrated any extensive penetration of these substances into the body of the rat. This may be due to the fact that the material does penetrate but is present in the body in amounts that cannot be detected by the chemical means that were used. This has been confirmed with experiments using P^{32}, I^{131}, Na^{24}, Ca^{45} and labeled diiodofluorescein iontophoresis, where the presence of these substances in various tissues of the body after iontophoresis has definitely been demonstrated. It has been shown that these isotopes, as illustrated by their presence in diverse amounts in various organs, enter into the body metabolism. For example, I^{131} introduced by iontophoresis appears in the thyroid gland in amounts greater than any other tissue which was examined. This obviously indicates a remote or systemic distribution.

Methylene blue dye, used in iontophoresis produced some systemic distribution. The presence of the dye in the urine after such experiments indicates this. Such a finding is definite evidence of a systemic effect. The presence of methylene blue in blood plasma was not detected in these experiments. Again this may be present in concentrations that were too small to be detected by the methods used.

Two cations; copper and mercury, two anions; iodide and borate, could not be detected in a similar manner by chemical means even though fairly sensitive chemical tests were used in the endeavor to detect them. Eosin, a negatively charged dye, also did not appear to be present in either blood plasma or urine.

In the case of the organic drugs which were used: strychnine, picrotoxin, nicotine and d-tubocurarine; all four of these positively charged chemicals produced clear cut pharmacodynamic evidence of their systemic distribution.

Using strychnine, it required a subcutaneous injection of two milligrams to produce responses which approximated that of the animal under iontophoresis in which a total of nine milligrams was used. We may estimate, therefore, that approximately 22 per cent of the applied strychnine was transported by iontophoresis.

With picrotoxin it required a subcutaneous injection of 2.4 milligrams to produce effects corresponding to those following iontophoresis where twelve milligrams were applied. Therefore, at least about 20 per cent of the applied picrotoxin appeared to be transported by iontophoresis.

With nicotine it required a subcutaneous injection of sixty-four milligrams to produce effects similar to those in the animal following iontophoresis where a total of 270 milligrams were used. Again we may therefore estimate that approximately 24 per cent of the applied nicotine was transported by iontophoresis.

With d-tubocurarine it required a subcutaneous injection of fifteen

units to produce responses in the rat similar to those produced following iontophoresis where a total of eighty units were applied. In this case it appeared that a minimum of about 19 per cent of the applied d-tubocurarine was transported by iontophoresis.

Gordon and Darling (1949) observed that the threshold values, to electrical stimulation of a suitable nerve muscle preparation, was not altered after iontophoresis with d-tubocurarine. Therefore, they believed penetration was limited. They used six rabbits in their experimental work. In the same year Neuwirth (1949) reported that curare solution was moved into the tissue of human patients by galvanic current. Such penetration was manifested by local vasodilation. This work did not report any effects on muscle activity.

The results reported here in regard to d-tubocurarine are also in contrast with those of Gordon and Darling. In our experiments the threshold values for electrical stimulation of the nerve muscle preparation of the rat and rabbit were markedly increased and finally abolished during iontophoresis with d-tubocurarine-(figure 5). In addition, depression of respiration and death ensued in some cases. This is a demonstration of the systemic action of d-tubocurarine introduced by iontophoresis.

Similarly the pharmacological response of generalized tetanic convulsions from the application of strychnine sulfate solution by iontophoresis is an indication of its systemic effect. A similar result had been reported by Leduc (1909). The pharmacodynamic response with nicotine sulfate was also an indication of its general systemic effect following iontophoresis. Picrotoxin produced a general systemic effect where convulsions were elicited after

its use by iontophoresis.

The only report in the literature where identification, in the remote tissues, was made of the substance used in iontophoresis was that of Inoue et al (1938). They obtained increased fluoride concentration in the blood using iontophoresis with hydrofluoric acid. Systemic distribution is clearly illustrated here. We have added the five radioactive substances used: P^{32}, I^{131}, Na^{24}, Ca^{45} and labeled diiodofluorescein and also the dye methylene blue.

The finding reported here, using iontophoresis with I^{131}, confirm and extend previous work in that the presence of iodine has been demonstrated not only in urine but in other body tissues. Using I^{131}, it was found to be deposited in various organs and tissues in a manner similar to that which would result if it were given by other routes of administration. The amount used in iontophoresis approximated 7.75×10^{-4} micrograms per milliliter. This helps to explain the fact that nonradioactive inorganic iodine could not be detected by chemical means. The total amount of the applied I^{131}, a fraction of a microgram, was too small even to be detected by ordinary chemical tests.

Likewise, iontophoresis experiments with P^{32} show the penetration of the material into the body systemically and deposition occured in a manner similar to that which would result had it been given by other body routes of administration—(Hevesy, 1948). Similarly, when Na^{24} was used, systemic effects were noted. Distribution of the isotope throughout the rat after iontophoresis, was obtained.

Diiodofluorescein, containing radioactive iodine, was demonstrated

to be present in various organs after iontophoresis. The rather low uptake by the tissues of this radioactive substance, probably can be attributed to the fact that it is a large molecule, much larger than the previous inorganic radioactive materials which were used. The lower uptake of radioactivity by the thyroid tissue in the rat can be probably explained by the fact that evidently there is little dissociation of the I^{131} from the fluorescein molecule.

Ca^{45} was also found to be present in various body tissues following iontophoresis. Its presence in comparatively high concentration in bone, after such experiments, indicates its systemic effect. The conclusion to be reached from the experiments that have been reported, especially those with the organic drugs and radioactive substance, is that such compounds under iontophoresis undergo sufficient penetration and distribution so as to produce a systemic effect. Such results lead us to compare iontophoresis effects to those following subcutaneous injection. The material is driven into the tissues and is slowly picked up by the capillaries and carried to the general circulation. Then it produces its pharmacodynamic response on the part of the body where it usually exerts such characteristic effects.

The question arose as to what charge the material should have for its optimal penetration into the tissue under iontophoresis. From experiments with dyes, methylene blue and eosin, we have obtained evidence of the significant penetration of methylene blue, a positively charged dye, while with eosin, a negatively charged dye, no such significant penetration was observed. It was thought that since the outside of a living membrane bears a negative charge, the repelling effect of this negative charge was responsible for lessened pene-

tration of the negatively charged dye used in iontophoresis. However, with the radioactive substances we have seen that this relationship does not seen to hold. I^{131} and P^{32}, negatively charged isotopes, show a larger per cent uptake by the tissues after iontophoresis than Na^{24}, a positively charged isotope. On the other hand Ca^{45}, a positively charged isotope, demonstrates a greater per cent uptake by the tissues after iontophoresis than I^{131} or labeled diiodofluorescein, negatively charged radioactive substances - (See table X and table XI). Therefore, it appears that there is no direct comparison between the charge of the isotope and the ability of its penetration into the tissues. At least this hypothesis seems to be indicated with experiments on radioactive substances.

It has been shown in experiments with reversal of poles (using the electrode which was opposite in charge to the material used in iontophoresis) that under these conditions the uptake of the material by the tissues is minute. This uptake with reverse poles compares very closely with the uptake by the tissues in experiments where the drug was applied to the "driving electrode" but no electric current was used. This constituted a control for our experiments. We can conclude that the penetration of the drug is very similar as far as these two experiments are concerned.

From purely physical and electrical considerations, the maximum movement of anions or cations is in the range of two centimeters per hour under a potential gradient of one volt per centimeter at 25° C. However, for organic materials, like the alkaloids, maximum ranges of movement are from 0.055 centimeters per hour under a potential gradient of one volt per centimeter.

It does not seem possible that direct electrolytic ion transfer, in the intact animal, could take place to any great extent. At least such transport does not seem likely to a sufficient extent to cause systemic pharmacodynamic effects by itself. We believe that the combination of introduction into the tissues by electrical current followed by dissemination by way of the circulation will explain the results obtained. Certainly we cannot agree with the assumption of Walzer and Golan (1946) that antigen is transported through a chain of five human bodies during twenty minutes of iontophoresis, by a solely electrical phenomemon. Of course some, as yet unexplained, biological mechanism may be operative. Our experiments do not offer any support to this "tissue plane" theory. The fact that tissues between the electrode, during iontophoresis, accumulate an amount of radio-isotopes, similar to other indifferent and remote tissues is positive evidence against such a theory.

We obtained certain evidence of general or systemic effects, especially with organic drugs which were used and with various radioactive substances. From these results we would say that in general it seems that local effects are bound to occur. We can hardly envision systemic effects without at the same time producing local area effects.

CHAPTER V

SUMMARY

1) It has been demonstrated that methylene blue dye may be present in the urine of the rat after appropriate iontophoresis with this dye. No methylene blue was found in blood samples or at distal sites, for instance the receiving electrode.

Dissection of the tissue near the "driving electrode" revealed that methylene blue dye had penetrated into the underlying tissue and into the bone to a total distance of about one centimeter.

2) Eosin dye, after iontophoresis, in the rat could not be detected in urine, plasma or receiving electrode samples. Dissection of the tissue near the driving electrode showed only faint and superficial staining. The staining appeared to be present for a distance of about one-half centimeter.

3) Inorganic ions, copper, mercury, iodine, and borate, were not detected by appropriate sensitive chemical tests, in blood plasma, urine and receiving electrode samples following iontophoresis with the individual ions listed.

4) Characteristic general pharmacodynamic responses were observed after iontophoresis with all four of the organic drugs which were used--strychnine sulfate, nicotine sulfate, picrotoxin and d-tubocurarine chloride.

5) By actual physical laws the average maximum range of movement of the anions and cations is in the order of magnitude of two centimeters per hour under a potential gradient of one volt per centimeter at 25°C. For organic materials like the alkaloids maximum ranges of movement are much less.

6) The presence of P^{32}, I^{131}, Na^{24}, Ca^{45} and labeled diiodofluorescein has been demonstrated in various tissues of the rat following iontophoresis. This distribution of the radioactive substances in the tissue resembles that obtained when isotopes have been given by other routes.

7) Reversal of the electrical pole at the "driving electrode" where the circuit pole was opposite in charge to that of the ions under investigation, using methylene blue, strychnine, nicotine, picrotoxin, d-tubocurarine, P^{32}, I^{131}, Na^{24}, Ca^{45} and labeled diiodofluorescein, resulted in little if any systemic penetration into the tissues of the rat.

8) By leaving the same substance in contact with the rat at the "driving electrode" and using no iontophoresis, a result was obtained which was similar to that obtained using the same substance and electrical circuit pole of reverse charge—namely little, if any penetration into the tissues.

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APPROVAL SHEET

The thesis submitted by Edward P. O'Malley has been read and approved by three members of the Department of Pharmacology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Date 4 Nov. 52

Y. T. O'Ester

Signature of Adviser