1954

Iontophoresis: Fundamental Experimental Studies Using Radio-Isotopes

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IONTOPHORESIS: FUNDAMENTAL EXPERIMENTAL STUDIES USING RADIO-ISOTOPES

by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June

1954

LIFE

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ACKNOWLEDGMENT

I wish to express my sincere thanks and appreciation to Dr. Y. T. Oester, under whose jurisdiction this research was carried on. His kind, attentive, and inspiring attitude helped to bring this research to a successful ending. It has been a rare pleasure indeed to have been associated with Doctor Oester during my graduate program at Loyola University.

This author is also indebted to Doctors Charles D. Proctor, Hugh J. McDonald, and Maurice V. L'Heureux, whose many valuable suggestions and generous assistance were a source of great help and encouragement in this research. Finally, appreciation is expressed to Doctor Frederick Benjamin for making available to us the original source of the tumor extract which we used in this study.

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CHAPTER I

INTRODUCTION

Iontophoresis may be defined as the process whereby substances in solution are applied to the surface of the body and introduced into the tissues by the use of electrical current. Electrophoresis is not included in this definition as it is a movement of a colloid (dispersed phase) in the presence of an electrical field with the dispersion medium held constant. In the same way our use of the term iontophoresis does not include electroosmosis which is defined as a movement of a liquid (dispersion medium) while the dispersed phase is held constant. Some of the literature have used these three terms interchangeably. However we wish to emphasize that in our opinion iontophoresis should be the only term applied to this general process. Ion transfer may be considered synonymous with our use of this term. There may take place some degree of either or both electrophoresis and electroosmosis 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 medical profession has been fascinated for more than a century by the introduction of drugs into the human body by electrical current. Turrell (1921) stated that such a form of medication was described as long ago as 1747 by Veratti. According to Jones (1907) Palaprat claimed to have introduced iodine in the tissues in this way in 1833. Frankenhauser (1906) in

1898 was very successful in introducing many drugs into the body by galvanic current. As a practical exercise in therapeutics, the general application of the theory must be credited to Ladus (1908) who brought it prominently before the medical profession, in a series of papers beginning in 1900. In our previous research study on iontophoresis, we obtained evidence for the penetration into body tissues of dye substances, organic drugs (Oester and O'Malley, 1953) and radioisotopes (O'Malley, Oester, and Warnick, 1954). We concluded in this study that iontophoresis is concerned with the introduction of a substance into living tissue by electrical current followed by dissemination by way of the circulation with a systemic effect thereby following.

Consideration of the problem led us to ask what influence physical factors such as pH, ionic strength, current strength, duration of iontophoresis, size of the electrode, concentration of the substance used, and physical state of the substance might play in iontophoresis. As we previously mentioned we believe that iontophoresis, or ion transfer by electrical current, produces initially an effect due to introduction, locally, of the substance into living tissue. Since this effect is an electrolytic one, as a physical phenomenon, it should be concerned with some of the physical chemical factors we have already mentioned. For this reason we decided to undertake in our present research an investigation of the possible effects of these various physical chemical factors mentioned on the process of iontophoresis.

We will review briefly the findings of other investigators who used iontophoresis, where they varied certain of these physical chemical factors.

1. pH

Abramson (1937), using iontophoresis into human skin with histamine, observed that variation of the pH of the solution at the active electrode demonstrated little, if any, effect on the physiological response measured by a wheeling reaction.

Harpuder (1937) concluded in his studies on iontophoresis that the pH of the electrolyte solution is important insofar as it increases or decreases ionization.

Harpuder (1938) in his discussion of the electrophoretic theory said that the introduction of positively charged substances from the positive pole in watery, neutral, or slightly alkaline solutions proved to be the most efficient procedure. He suggested that a negatively charged solution be introduced from the negative pole in acid solution.

Bierman (1938) considered pH a factor in the use of iontophoresis in human subjects as a therapeutic measure. He also advised that, for greatest effectiveness, positively charged substances be introduced in neutral or slightly alkaline solutions and negatively charged substances in acid solutions with a pH of less than three.

2. IONIC STRENGTH

Abramson (1937) noted in iontophoresis with histamine into human skin that by increasing the ionic strength (using KCl) at the active electrode there was a decrease in the effectiveness of histamine transfer into the skin. The effectiveness of iontophoresis was titrated by measuring the extent of the wheeling effect produced by histamine.

3. CURRENT STRENGTH AND DURATION

Many of the research workers have reported findings on the influence of current strength and time of application on the effectiveness of iontophoresis. Abramson (1942) noted in his experiments with mecholyl iontophoresis in human patients that there was no clear cut evidence obtained to indicate that a longer period of application elicited a greater vasodilator response than the one of shorter duration. Similarly, a higher current density was no more efficient than the lower one.

On the other hand Fette (1918) noted in his experiments using iontophoresis with zinc that current strength and time of iontophoresis were factors in the total transfer of material by this method. Friel (1918) also indicated that the time of iontophoresis and current strength were important factors in such iontophoresis. Blackmar (1928) postulated in his experiments, where he employed zinc iontophoresis in treatment of purulent otitis media, that the electric current furnishes a "pressure" which carries antiseptics inwardly through the skin and mucous membrane. Further, the depth of penetration, within limits, depended on the strength of the current used and the duration of its use. Walker (1933) employing iontophoresis with zinc in humans mentions that the distance the zinc penetrates into the tissue depends on the strength of the current, the length of treatment, and the kind and nature of the tissue to be treated. Wells (1942) stressed the importance of estimating the quantity of electricity used in the process in treatment of chronic suppurative media.

Bredall (1939) found that the amount of copper driven into the tissues by iontophoresis was in direct proportion to the amount of current, and the length of time the current was flowing. He commented further that a

longer treatment with a relatively small amperage will drive the copper ions deeper into the tissues than a short treatment. Too high an amperage caused too great a coagulation of the tissues and hindered the movement of copper ions.

Sloan (1911) in the use of iontophoresis with organic drugs and metals, for treatment of certain gynecological diseases, took into account the coulombs of electricity which were used. He tried to calculate the amount of a given material penetrating into the tissue. Indirectly he supported the hypothesis that the current strength and duration of iontophoresis was directly related to the penetration of a substance into tissue under the influence of electrical current. Pack et al (1924) concluded after his iontophoresis experiments using organic drugs and metals that the number of migrating ions is dependent on the quantity of current applied. He continues that the migrational velocity is important, nevertheless, in the passage of the current, since the quicker moving ions convey the greater part of the current, e.g., hydrogen ions carry five times the quantity of electricity that is carried by the chlorine ion.

Kovacs (1934) in iontophoresis experiments using acetyl-beta-methyl-choline-chloride in the treatment of chronic arthritis and peripheral vascular disease in humans indicated that the factors governing the depth of penetration were (1) length of time current flows, (2) the electromotive force, and (3) the tissues being treated. Kotkis et al (1935) found after the introduction of acetyl-beta-methyl-choline-chloride by iontophoresis in dogs, with a current density of 10 to 50 milliamperes, there was observed a consistent lowering of blood pressure (10 to 65 mm.). In the majority of experiments, the rapidity in the drop of blood pressure and the length of time it was maintained varied

directly with the intensity of the current. Bredall (1938) noted in the treatment of arthritis with mecholyl iontophoresis in humans that the rapidity of action and the duration of effect can be fairly well controlled by the amount and the duration of the current applied. Montgomery (1938) observed after iontophoresis in dogs, using acetyl-beta-methyl-choline-chloride, that the extent of the fall in blood pressure varied directly with the amperage of the current and that the effect was due to the drug rather than to the passage of the current alone, since it was absent when saline was substituted for the drug. The author generally confirmed the results of Kotkis et al. Molitor (1943) observed in a large number of experiments recording carotid blood pressure in the cat, after iontophoresis with mecholyl, that the general effects of the drug appeared within a relatively short time, depending largely on the amount of the current flow. Macht et al (1948) found in human subjects that with iontophoresis of acetyl-beta-methyl-choline-chloride when the intensity of the current was increased from 10 to 18 milliamperes there resulted greater increases in blood flow. However there was some variation in this, since one human subject reacted as strongly to 10 milliamperes as he did to 18 milliamperes.

Kling (1935) noted that when histamine iontophoresis is prolonged or when the current density was increased, general systemic reactions occurred. Kling et al (1937) reported essentially the same findings. Abramson (1949) found, in several human patients using histamine iontophoresis for multiple sclerosis, that either increasing the current strength or time of iontophoresis produced an increased physiolgical response. This was noted by the primary flush reaction remaining for a longer period of time after treatment had begun.

Rodriquez et al (1950) found that the average blocking of the wheal effect, using an adrenergic blocking agent administered by iontophoresis, was found to be 83 per cent at the five minute time level and 54 per cent at the four minute level. This indicates that the duration of iontophoresis had a pronounced effect on the total transfer of this material. Also it was found that the extent of activity of the adrenergic blocking agent is a function of the duration of iontophoresis. This work was done on human patients.

Boyd (1942) attempted to determine the optimum strength of current and time of application of sodium sulfathiazole by iontophoresis in rabbits. He thus varied these factors independently. With a current of 2 milliamperes for two, five, and ten minutes it was found that the concentration in both the cornea and the aqueous humor varied directly but not proportionately with the time of application. With a variation in the strength of the current of 1, 2, 3, 4, and 5 milliamperes and a constant application time (five minutes) there was again a direct but not proportional variation in the sulfathiazole content of the cornea and the aqueous humor.

Pereyra (1948) applied penicillin by iontophoresis in the treatment of chancroidal ulcers in human patients. He found that the amount of penicillin recovered in the urine varied directly with the intensity of the current used and the duration of the treatment.

Von Sallmann (1943) observed that by increasing the current strength and length of iontophoresis applied to the eyes of rabbits there resulted an increased amount of atropine in the aqueous humor as compared to the lower current density and shorter duration.

Abramson (1939) observed that the electrophoretic method not only

administers small quantities of ragweed intradermally, but also permits different quantities to be administered by varying the current strength. His work was done on human patients. Further, (1941) in his experiments with giant ragweed, dwarf ragweed, and timothy extract he found that by increasing the current density and maintaining the duration of iontophoresis constant a similar result was produced to that when the duration of the current was varied and the current density was kept constant. Shilkret (1942) noted that iontophoresis with grass extracts in human patients produced a reaction that could readily be altered by changing the current density and the duration of iontophoresis.

Strohl et al (1950) demonstrated with radioactive iodine and strontium that as iontophoresis was prolonged the activity found in the blood of different rats was a function of the intensity of the current and the duration of the application. The activity of the radioactive material in the blood tapered off after iontophoresis was discontinued.

4. ELECTRODE SIZE

Montgomery (1938) observed, after iontophoresis in dogs using acetyl-beta-methyl-choline-chloride, that variation in the electrode size of the positive pole over a wide range did not affect the degree to which blood pressure was reduced by given strengths of the drug. Molitor (1942) noted in blood pressure experiments in cats, where mecholyl iontophoresis was employed, that neither the size of the electrode nor the skin resistance at the site of application influenced the result, if equal currents were used for an equal length of time.

Abramson (1941) in his experiments with giant ragweed, dwarf ragweed,

and timothy extract iontophoresis, found as long as the current density was kept constant the electrode area could be increased without altering the amount of material that was introduced.

Wolf (1933) found with iontophoresis in human subjects that the greater the resistance the longer the application should be, or the greater the amount of current which must be applied. However, no data was given to support his claim.

Bredall (1939) found that the amount of copper driven into the tissues by iontophoresis was in direct proportion to the size of the electrode used.

Abramson (1939) found that the quantity of ragweed introduced intradermally by iontophoresis could be changed by varying the electrode area.

5. CONCENTRATION

Fette (1918) postulated that the quantity of ions introduced by iontophoresis is proportional to their chemical equivalents and independent of their concentration. Pack et al (1924) quotes Hittorf as finding that the concentration of the solution is the most important factor. He states that the greater the degree of dissociation of the solute, the greater the facility with which the current travels, since the conductance of a solution is due in part to the dissociated portions of the molecules.

Kovacs et al (1936) found in their experiments with acetyl-beta-methyl-choline-chloride in humans that essentially the same physiological actions were produced with two different concentrations of this choline compound. In one case they used a 1 per cent solution and in another a 0.5 per

cent solution. Bredall (1938) noted in the treatment of arthritis with mecholyl iontophoresis in humans that the concentration of mecholyl in solution within limits from 1-200 to 1-8000 did not seem to influence the physiologic effects produced.

Kotkis et al (1935) found in iontophoresis experiments with dogs using acetyl-beta-methyl-choline-chloride in dilutions ranging from 1-1000 to 1-10,000 that the results obtained with the several dilutions showed very litt change except with the dilution of 1-10,000 where a decided difference in blood pressure was observed. Therefore he was led to believe that the increasing dilutions of this drug, within the limits investigated, do not result in lesser physiological effects when the drug is administered by iontophoresis. Boyd (1939) questioned the results of Kotkis regarding the physiological effects obtained with two different concentrations of acetyl-beta-methyl-choline-chloride. He mentioned that in his work decided difference was found between the effects obtained with a 1-100 and a 1-200 solution of this drug introduced by iontophoresis. The weaker solution gave none of the clear-cut general reaction obtained with the larger concentration. Molitor (1943) noted in a large number of experiments recording carotid blood pressure in a cat that the use of a 0.5 per cent or a 1 per cent solution of mecholyl, administered by iontophoresis, resulted in greater changes than when a 0.1 per cent solution was used.

Abramson (1937) observed with iontophoresis into the human skin with histamine that the concentration of the material had a definite effect on the local response to this process. Whereas 1:100,000 dilution of histamine introduced by iontophoresis produced a small local whealing reaction, a 1:5000 dilution of histamine produced a large whealing effect. The current density

was maintained constant during the experiment. Abramson (1949) showed that by increasing the concentration of epinephrine, given concomitantly with histamine into the human skin by iontophoresis there resulted a greater inhibitory effect against the action of histamine (marked by whealing of the skin) than was found when a lesser concentration of epinephrine was used.

Von Sallman (1944) found that the iontophoretic introduction of a 0.25 per cent solution of sodium penicillin into the aqueous humor of rabbits led, in forty-five minutes to a maximal concentration of 40 micrograms per cubic centimeter. The average for this higher concentration was about three times as great as that obtained with a solution of 0.1 per cent sodium penicillin and almost ten times as great as the average concentration in the aqueous humor forty-five minutes after a single corneal bath with a 0.25 per cent solution without electric current. Pereyra (1948) observed that the amount of penicillin recovered in the urine after being introduced by iontophoresis in the treatment of chancroidal ulcers in human patients varied directly with the concentration of the penicillin solution used at the active electrode.

Erlanger (1939) noted in his experiments using prostigmine iontophoresis into the eyes of rabbits that the higher the dilution of prostigmine the longer is the latent period of the pharmacological response—the miosis setting in much later.

Von Sallman (1943) employed various concentrations of atropine sulfate solution in the iontophoresis of these solutions into the eyes of rabbits. He observed that when 0.1 per cent atropine solution was used, the amount of alkaloid in the aqueous humor, estimated by bioassay, was six times more in the

eyes treated by iontophoresis than in the control eyes where the same solution was used as an eye bath. When a 0.25 per cent atropine sulfate solution was used, the concentration in the aqueous in the eyes exceeded by nine times that in the control eyes.

Abramson (1939) observed that the quantity of ragweed introduced by iontophoresis is dependant somewhat on the concentration of the solution used at the active electrode. Shilkret (1942) noted that the intensity of the reaction produced by iontophoresis with grass extracts in human patients may be readily altered by changing the concentration of the solution used at the active electrode.

Harpuder (1938) in his discussion of the electrophoretic theory concluded that in iontophoresis the highest possible concentration of the ion to be introduced is advantageous.

6. PHYSICAL STATE

As another physical chemical variable, the physical state of the substance used in iontophoresis becomes an important consideration. There is an apparent lack of experimental studies regarding a comparison of the physical states of the materials introduced by iontophoresis. In this regard, experiments were undertaken to compare the effect of the iontophoretic introduction of various colloids to selected non-colloidal materials.

In the literature there are no extensive reports where attempts were made to organize and summarize the available experimental work concerning these studies. Such a survey has been made here. Table I lists the findings of this survey.

Because there was a wide divergence of opinion concerning the influence of various physical chemical factors on the iontophoretic process and since the experiments reported in the literature were, in general, not adequately controlled and in some instances deficient, we decided to undertake a detailed, controlled, experimental approach to this problem, using an essentially quantitative method by employing radioisotopes.

TABLE I (CONTINUED)

IMPORTANT REPORTS ON PHYSICAL CHEMICAL FACTORS IN EXPERIMENTAL IONTOPHORESIS

Author	pH	Ionic Strength	Electrode Size	Conc. of Substance	Current	
					Strength	Duration
Kovacs et al (1934)					+	+
Kovacs et al (1936)				0		
Macht et al (1948)					+	
Molitor (1943)			0	+	+	+
Montgomery et al (1938)			0		+	
Pack et al (1924)				−	+	+
Peroyra (1948)				+	+	+
Rodriquez et al (1950)						+
Shillcrat (1942)				+	+	+
Sloan (1911)					+	+
Strahl et al (1950)					+	+
Von Sallman (1943)				+	+	+
Von Sallman (1944)				+		
Walker (1932)					+	+
Wells (1942)					+	+
Wolf (1933)			+			

* + = positive effect
0 = no effect
− = negative effect

TABLE I

IMPORTANT REPORTS ON PHYSICAL CHEMICAL FACTORS IN EXPERIMENTAL IONTOPHORESIS

Author	pH	Ionic Strength	Electrode Size	Conc. of Substance	Current	
					Strength	Duration
Abramson et al (1937)	0	+		+		
Abramson (1939)			+	+	+	
Abramson (1941)			0		+	+
Abramson et al (1942)					0	0
Abramson (1949)				+	+	+
Blackmar (1928)					+	+
Boyd et al (1939)				+		
Boyd (1942)					+	+
Bierman (1947)	+					
Bredall (1938)				0	+	+
Bredall (1939)			+		+	+
Erlanger (1939)				+		
Fette (1918)				0	+	+
Friel (1918)					+	+
Harpuder (1937)	+					
Harpuder (1938)	+			+		
Kling (1935)					+	+
Kling et al (1937)					+	+
Kotkis et al (1935)				+	+	+

IONTOPHORESIS AND ROUTES OF INJECTION

Literature reports indicate that iontophoresis produces a more diffused penetration of a given material into the local tissues than other methods of local injection. The support for this statement is made mainly on the basis of a particular physiological effect after treatment with iontophoresis--such as the prolongation of a physiological response after iontophoresis as compared to the effect produced after the introduction of material by other routes of administration. In general selected physiological effects were taken as the criteria rather than the determination of the amount of a substance introduced into the local tissues. Because there was no direct comparison made in the literature between injection studies and iontophoresis, relative to local concentration of introduced material, we decided to investigate this aspect of the problem more completely with the use of radioactive isotopes.

However, a few research workers had published reports where chemical and physical means were employed to demonstrate the presence of a substance after iontophoresis. Inchley (1921) detected iron by chemical means in skin and muscle of the rabbit after its introduction by iontophoresis. He also found salicylate in the urine of the cat 25 minutes after iontophoresis with this substance. The author suggested that the depth of penetration indicated that the action of the current increased the rapidity of absorption of the introduced substances along identically the same channels as in the controls, namely, as directly as possible from the skin to the perivascular lymphatics which drain the skin layers. Bourguignon (1922) introduced iodine in the form of potassium iodide by electric current and recovered iodine in the urine of human patients. Strehl et al (1950) were the only workers who have reported

the use of iontophoresis with radioactive isotopes. These authors demonstrated the presence of radioactive iodine in the blood of rats, guinea pigs, and rabbits after iontophoresis by counting procedures employing the Geiger Mueller Counter.

The local tissue deposition of a given material was indicated early in the inception of iontophoresis as a form of therapy. Clark (1919) observed that by iontophoresis larger quantities of a drug can be applied directly in any given localized area than would be possible by other methods of administration; the introduction of the drug is more rapidly effected, and a more prolonged action is secured. Rolfe (1919) noted that the substance introduced as ions by an electric current remains much longer in the tissues than when injected hypodermically. In the latter case, he postulated, they occupy the interstices of the connective tissue and are quickly carried off in the lymph stream, whereas, when introduced by electric current, they enter into the actual cells of the tissues and are more slowly eliminated. A study of the literature where a comparison was made between the effects produced by iontophoresis and that produced by other routes of administration is summarized in the accompanying table, taken from the work of Kovacs & Kovacs (1934).

STUDY OF EFFECTS OF DIFFERENT METHODS FOR ADMINISTERING SELECTED VASODILATING DRUGS

Route	Mecholyl	Acetyl Choline	Histamine
Oral	Mild general effect 1/2 - 1 hour Dose 100 - 200 mgms.	None	None
Subcutaneously	Powerful general effects lasts 15-20 minutes Dose 5-25 mgms.	Mild general effect lasts 15-20 minutes Dose 100-200 mgms.	-
Intravenously	Pronounced general effect lasts 20-40 minutes	Mild general effect lasts 10-20 minutes	No general effect
Iontophoresis	Pronounced local effect lasts 4-10 hours Dose 0.5-1% solution	Mild local effects lasts 1-2 hours Dose 0.5-1% solution	Pronounced local effect 2-4 hours Dose 1:20,000 solution

TUMORS

A more recent approach to local deposition of introduced material was that taken by the present author (O'Malley, Oester, and Warnick, 1954) using iontophoresis with radio-isotopes. In this work the amount of material entering the local tissue was estimated by radioactive counting of the local tissue area. Since our early results had borne out the reports of previous research workers in this field, that iontophoresis produces a more diffused penetration of the material into the local area, this technique was suggested for possible use in sub-surface tumor therapy. Injection techniques are presently employed in sub-surface tumor therapy. This injection form of therapy suffers the drawback of not delivering an adequate amount of radiation

(employing radioactive materials) or cancericidal material to all of the tumor cells involved. Therefore we postulated that iontophoretic introduction of a substance might show a more intense distribution in the local tissue. For this reason we decided to attempt to evaluate the usefulness of iontophoresis in tumor therapy.

The Walker rat tumor was employed in these studies. As a matter of history, Earle (1935) reported that in 1928 Dr. George Walker observed a tumor on the lower left side of a well grown female rat apparently ten months of age. Histological study confirmed this to be a carcinoma. Transplantation of this tumor was successful and now, 26 years and many, many transplants later, this transplantable tumor is still being used. Schrek (1935) in a quantitative study of the Walker rat tumor illustrated growth curves and gave directions for injecting the tumor. Talalay et al (1952) summarized various factors that contributed to the variability in the growth rate of the transplantable tumor.

Reports in the literature relative to the use of iontophoresis in tumor therapy appear rather scanty, according to a survey made by this writer. There are, however, a few that are related to this subject. Clark (1919) reported the removal of multiple warts by application of magnesium iontophoresis. Wardle (1919) observed the disappearance of a cancer of the rectum subsequent to iontophoresis with zinc using a current of 60 milliamperes for thirty minutes on alternate days. Borrel et al (1922) treated implanted sarcoma in rats by fixing positive carbon electrodes over the tumor on compresses saturated with various metallic solutions. Curative results were reported in a number of instances, especially with the lead ion. Pack et al (1924) summarized the work on tumors with iontophoresis by saying that the metals deposited on the surface of

the tumor were somewhat corrosive, but the destructive action was probably not due to this alone. Referring to the above protocol, he noted that even seven milliamperes of current produces a coagulative phenomena. A milliamperage of 60 would be quickly destructive; liquefaction occurs at the negative pole; coagulation at the positive. These facts lead Pack et al to believe that the beneficial results obtained in the treatment of tumors, and the various lesions mentioned, were due in large part to the action of the electric current per se.

It is well known that radioactive isotopes have proven effective in therapy against localized tumors. In the choice of a suitable material several criteria have been employed. These are listed as follows:

1. It must be composed of physiologically harmless elements.
2. The particle size must be large enough to prevent dissemination.
3. It must be stable enough to prevent uneven local precipitation.
4. It must be available in high specific activity so that abnormally large volumes are not required.
5. It must have a relatively short half-life so that the whole of the dose is delivered in a reasonably short time.

Colloidal Au^{198} appears to fulfill these requirements more adequately than other radioactive materials. Therefore we chose this substance in our studies on the evaluation of iontophoresis in tumor therapy.

Briefly we will summarize some literature reports indicating the employment of radioactive colloids in their experimental procedures. Sheppard et al (1947) found when colloidal sols of manganese dioxide and gold were administered intravenously in human beings and dogs there were variations in between liver and spleen regarding their content of radioactivity. In spite of

such variations he found, however, the picture was consistent in that liver and spleen are roughly equivalent in concentration in radioactivity and outstandingly high compared to other tissues. They utilized both 30 minute and 24 hour samples, after introduction of the radioisotopes. Bertrand et al (1948) traced the distribution of radioactive gold thiosulfate in the various tissues of the rat, taken 2 to 8 days after injection. They found that the kidney, spleen and liver seemed to have the greatest concentration of this material. In rabbits a similar distribution pattern was found 17 days after injection. Goldie et al (1950) withdrew specimens of peritoneal fluid from tumor bearing mice of three consecutive days following treatment with radioactive gold by intraperitoneal injection. Stained smears from these specimens revealed the absence of tumor cells or cytological abnormalities therein, in gold treated mice, while in the controls the tumor cells were numerous and showed a high proportion of mitoses. Thus he described the complete destruction of free tumor cells in the peritoneal cavity of the mice as a selective radio-therapeutic effect of radioactive colloidal gold. Sherman et al (1950) in the experimental application of radioactive colloidal gold by infiltrating it around the edges of a pelvic cancer, found very little mobilization of the material from the injected local site. Most of the radioactive gold remains in the local tissue, with the spleen and liver picking up slight amounts of it in the rabbit. The authors used the radioactive gold as an interstitial therapy method, in the treatment of transplanted squamous cell carcinoma in mice. It was found that these tumors could be safely and completely cured, with high survival rates, using this therapy. Berg (1951) demonstrated the presence of colloidal radio-

active gold in various tissues of the dog; such tissues were taken 2.7 days after intravenous, intraperitoneal, and intra-arterial injection. The gold was found most concentrated in the liver and spleen with all of these administration routes. In the human intravenous injection resulted in similar distribution patterns. Hahn et al (1951) carried out intraperitoneal injections of from 0.1 to 0.75 mc. of radioactive colloidal gold into mice 3 or 4 days after inoculation with leukemic cells and found that this treatment increased the life span by 7.5 to 38.5 per cent over that of untreated controls. Wheeler et al (1951) injected fourteen dogs intravenously with 1 mc/kg. of colloidal Au^{198}. All of the dogs showed a definite decrease in white count, but only one was thrown into marked leucopenia. In 13 out of 14 dogs the sedimentation rate increased following the treatments, although this response was not as clear cut as the decrease in white count. Hemoglobin and hematocrit decreased in a slow and progressive manner over a period of several weeks. Liver-function tests failed to show any marked degree of hepatic impairment. Walton et al (1952) stated that the value of intracavitary irradiation using radioisotopes as a curative method could not be assessed until a much larger series of cases were under observation. However, on the basis of this report it appears that conventional radio-therapy, either alone or in combination, has strong potential possibilities in use as a therapeutic measure against tumors. Wheeler et al (1952) noted that in injection techniques of tumors, using radioactive gold, the size of the tumor and the number of equivalent roentgens to be delivered to it will decide the volume of each injection. He defined the equivalent roentgen as that amount of beta radiation which under equivalent conditions released in one gram of air as much energy as one roentgen of gamma rays. He suggested

that the volume of fluid injected should approach the maximum.

In brief recapitulation of the general program of this subject, it is the purpose of the present report to encompass experiments on the following:

1. To vary factors such as pH, ionic strength, current strength, duration of iontophoresis, size of the electrode, concentration of the substance, and physical state of the substance used in an effort to observe the effect of these conditions on the introduction of a particular substance by iontophoresis.
2. To make a detailed comparison between subcutaneous, intraperitoneal, intramuscular, intravenous injections, and iontophoresis, by a tissue distribution study comparing their patterns of tissue distribution.
3. To introduce radioactive gold by iontophoresis in a study of the possible usefulness of this technique on sub-surface tumors.

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 vegetables and fresh meat at least once a week. The weight range of the animals was between 150 and 300 grams, with the exception of one small group where 75 gram rats were used. Routine stock guinea pigs and rabbits were also used to a limited extent in this research.

Pentobarbital anesthesia was used for all experiments. It was prepared by taking one gram of pentobarbital powder and dissolving it in 100 milliliters of distilled water. The dosage range for the rat was thirty to fifty milligrams of pentobarbital per kilogram, intraperitoneally. For the guinea pigs, fifteen milligrams of pentobarbital per kilogram, intraperitoneally, was used. In rabbits 30 milligrams per kilogram intraperitoneally was employed.

The iontophoresis apparatus consisted of a Goleth Fissell Constant Current Generator, as the current source. The electrodes consisted of platinum wire terminals bound around a piece of moistened cotton in contact with the body surface of the rat. Platinum wire was used, as it was thought that the degree of dissociation of the metal into the electrode fluid would be minimized by this type of material. 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. The "receiving electrode", the opposite pole, was attached to the rear right foot of the rat, approximately twenty centimeters from the driving electrode, point of application of the material under study. The cotton of the "receiving electrode" was moistened in all cases. The experimental arrangement for iontophoresis in the rat is illustrated in Figure 1. In most experiments, the "driving electrode" and the receiving electrode" each represented an area of approximately six square centimeters. A piece of glass tubing was slipped over the electrode area on the left upper arm and sealed with paraffin at its innermost end. This served to prevent any superficial contamination of the adjacent tissues when the active material was applied. Appropriate aqueous solutions of the materials to be used were carefully applied to the cotton through the open end of the tube. The same general set-up was employed with iontophoresis experiments in the guinea pig. In general, three rats were employed for each experimental series.

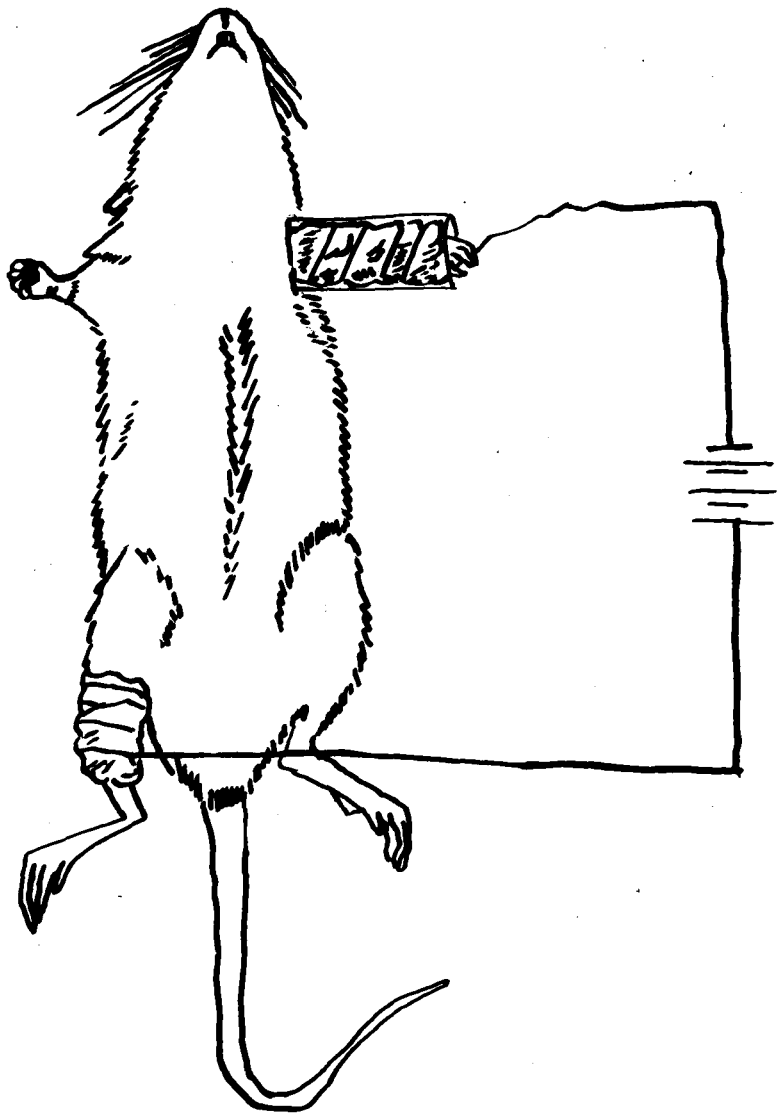


FIGURE 1. CONVENTIONAL ARRANGEMENT USED IN IONTOPHORESIS

Iontophoresis experiments in rabbits were made using cup shaped electrodes. These were made out of small aluminum planchets (2.5 cm./diameter) with a platinum wire soldered to its bottom. Cotton pads were inserted into the cavities of the planchets, and were in contact with the body surface. A small hole was also punched into the bottom of the planchet so that the materia to be introduced by electrophoresis could be added directly through this aperture after the electrodes had been placed on a desired site. The electrodes were held at the desired site by a paraffin seal. In the rabbit, one electrode was placed over the chest area ("driving electrode") and the "receiving" electrode was placed over the lower abdominal area.

The cup shaped electrodes were also employed for tumor studies. In some experiments the electrodes were placed on opposite sides of the tumor and in others only one electrode was placed over the tumor and the other placed over an indifferent area, usually over the chest region. One type of placement is illustrated in Figure 2. In the tumor bearing rats, after iontophoresis, the tumor tissue was divided into three sections: the area nearest the "driving electrode", the portion nearest the "receiving electrode", and the middle section.

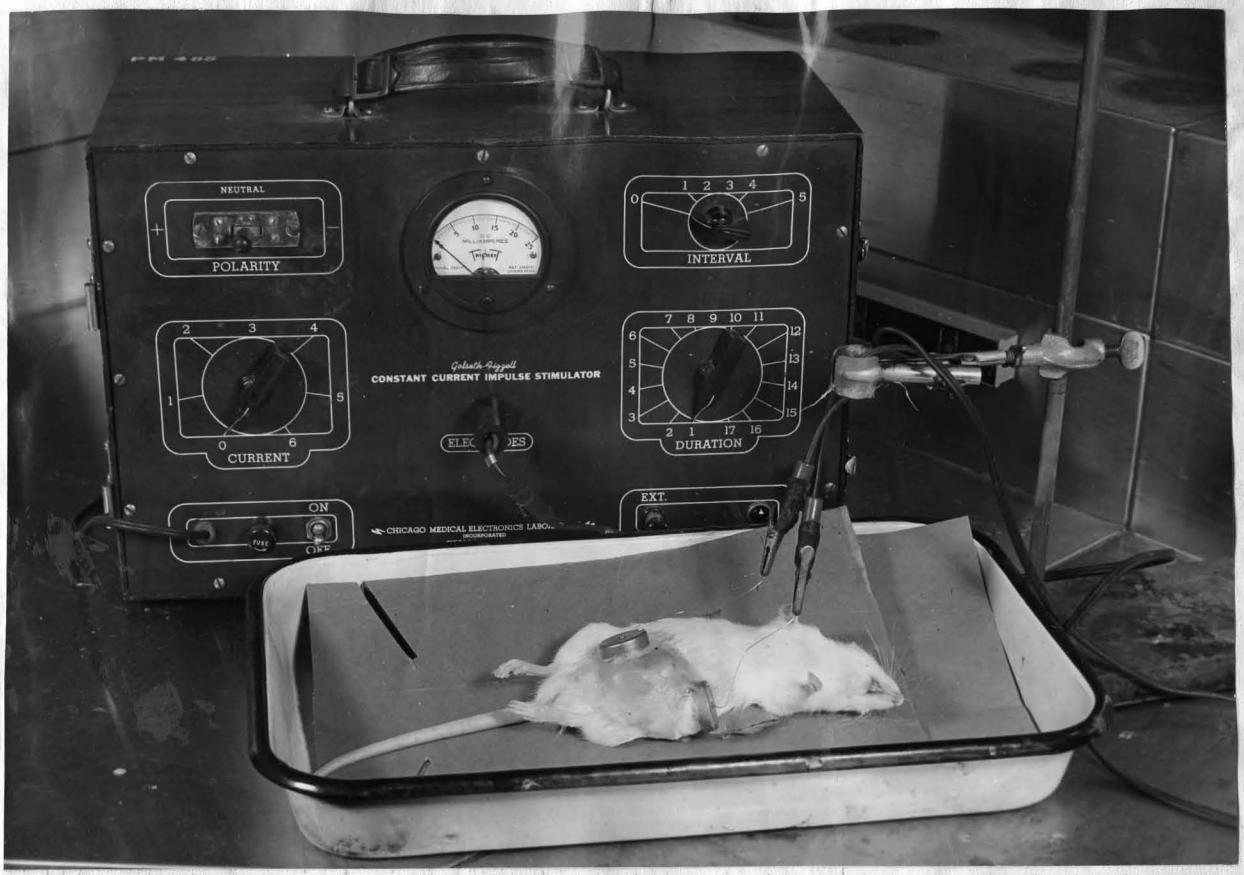


FIGURE 2. IONTOPHORESIS ARRANGEMENT USED WITH RAT TUMORS

Forty-eight hours before each experiment, the hair was removed from the electrode sites, by a depilatory formula containing barium sulfide, detergent, and hydro-alcoholic solution, devised for use in laboratory animals, Pitesky and Last (1948). The depilatory formula was prepared by triturating two-thirds by weight of purified yellow barium sulfide powder with one-third by weight of a commercial detergent. We used "Cheer" (Procter and Gamble) and "Tide" (Procter and Gamble) in our experiments. Twenty-five grams of the depilatory mixture were mixed with 50 milliliters of a 10 per cent glycerine-in-water solution until a smooth creamy suspension was obtained. The area to be treated was thoroughly wetted down with water. The depilatory was then applied with a wooden tongue-depressor blade and gently worked into the hair. When the hair had been completely removed, the area was rinsed off with a copious amount of water to insure complete removal of any sulfide residue. A period of forty-eight hours was allowed to elapse between removal of the hair and the iontophoresis, so as to minimize the possibility of skin irritation contributing to the facility with which the material penetrated the tissue during iontophoresis. However, there did not appear to be any significant irritation (as shown by reddening of the skin) immediately after removal of the hair by the depilatory. This type of hair removal was found to be superior to shaving or clipping, as it removes hair more completely, is more rapid, and produces minimum trauma.

RADIOACTIVE SUBSTANCES USED IN IONTOPHORESIS

Radio-isotopes used were obtained from the Oak Ridge National Laboratory or from Abbott Laboratories. The original radioactive isotopes were in solution. In all instances, a 0.1 milliliter aliquot of the supplier's original solution was diluted to 100 milliliters with distilled water and radioactivity counts were taken on this diluted sample. This was used as an index for determining how much radioactivity (counts/minute) was applied at the "driving electrode". The quantity of radioactivity (counts/minute) applied at the "driving electrode" was used as a basis for calculating the per cent uptake of the material by the animal. In general, an aliquot from the 1:100 dilution of the solution (as received from our suppliers) was employed at the "driving electrode" site, or for the selected injection procedures.

(a) P^{32}

Radioactive phosphorus was received in the chemical form of a phosphate in weak HCl. It had a specific activity of 0.025 mg. P/mc. P^{32}. The fluid dilution of this material resulted in approximately 35 - 60 microcuries in two milliliters of distilled water at a pH of 5.0. This amount was used at the "driving electrode", the cathode, in the manner already described. With the concentration studies, lesser amounts of radioactivity were employed.

(b) Ca^{45}

Radioactive calcium was received in the chemical form of $CaCl_2$ in weak HCl with a specific activity of 11.65 mc./gm. Ca. Approximately 350 microcuries of Ca^{45} in two milliliters of distilled water at a pH of 4.0 was placed on the "driving electrode", the anode, in the manner already described.

(c) I^{131} Labelled Diiodofluorescein

Approximately 100 microcuries of labelled diiodofluorescein in the form of sodium salt, in two milliliters of aqueous solution at pH 7.9 were placed on the "driving electrode", the cathode, in the manner already described. The specific activity was not stated on the invoice slips arriving with this radioactive material from our supplier, and subsequent investigation was not successful in attempting to find what it was.

(d) I^{131} Labelled Albumen

Approximately 200 microcuries of labelled albumen in two milliliters of aqueous solution at pH 7.0 were placed at the "driving electrode", the cathode, in the manner already described. The particle size of a molecule of albumen, as calculated by this author (from the data of West et al, (1951) was approximately 0.0275 microns. It was not possible to find the specific activity of labelled albumen from the information supplied.

(e) Colloidal Au^{198}

Approximately 234 microcuries of Au^{198} in the colloidal form in two milliliters of an aqueous solution at pH 4.0 were placed at the "driving electrode", the anode, in the manner already described. The particle size of a molecule of colloidal Au^{198}, as indicated in the brochure from our supplier, was approximately 0.003 microns. Again, it was not possible to find the specific activity of this material from the information supplied.

SAMPLES FOR RADIOACTIVITY MEASUREMENT

The radioactivity of all of the samples was measured in the liquid

state, employing the method described by Friedman and Hume (1950). Each of the test samples was measured by counting the treated sample with an unshielded end window Geiger Mueller tube and autoscaler combination. The window thickness of the Geiger Mueller Counter tube that we employed was 2.2 mg./cm^2. A comparison sample, which represented a suitable aliquot of the original radioactive solution was used as the standard. This eliminated the need for calculation of radioactive decay.

After the iontophoresis application, a series of samples were taken from the animal for assay for radioactivity. Such samples were usually taken from the blood, muscle below the "driving electrode" area, kidney, liver, indifferent thigh muscle, indifferent thigh bone, and urine. In certain cases, spleen samples were also taken. The various samples taken weighed from 0.3 to 0.9 grams, depending on the particular tissue. The blood of the animals was taken by cardiac puncture, one milliliter of blood being collected each time. In the experiments where P^{32}, Ca^{45}, and Au^{198} were used, five milliliters of 10N sulfuric acid were added to the various weighed tissue samples for digestion purposes. Gentle heat was applied until complete solution was obtained. The samples were then decolorized with a small portion of thirty per cent hydrogen peroxide, so as to insure homogeneity of the final solution. Each sample was diluted to ten milliliters in a volumetric flask. A one milliliter aliquot from this dilution was taken, placed in a porcelain capsule of four centimeters diameter, and radioactivity counts were taken. The number of counts was generally around 25 times that of the background. When diiodofluorescein labelled I^{131} and albumen labelled I^{131} were used, the samples were treated as described, except thirty per cent potassium hydroxide was substi-

tuted as the digesting agent in place of sulfuric acid.

NON-RADIOACTIVE MATERIALS USED IN IONTOPHORESIS

(a) Strychnine Sulfate

Three different concentrations of strychnine sulfate were used in the iontophoresis experiments--a 0.5 per cent solution, a 1.0 per cent solution, and a 2.0 per cent solution. The final strychnine solution contained a total ionic strength of 0.1 with the use of NaH_2PO_4 in the three solutions. The pH was 5.0 per cent in each solution also. Two milliliters of the solution were applied to the "driving electrode", the anode, with all experiments. Five milliamperes of current for one hour was applied in all cases, as the period of application of iontophoresis.

(b) Insulin (Iletin-Lilly)

An insulin solution was prepared by taking Insulin and diluting it to such volume so that the solution contained 30 units of insulin per milliliter. This insulin solution was brought to the acid side of the isoelectric point of insulin with HCl so that the final solution was at a pH of 3.0. Two milliliters, or 60 units of this solution were applied at the anode (positive pole). Five milliamperes of current for one hour was used in all experiments with this material.

The size of the insulin molecule was estimated to be in the order of 0.005 microns, according to West et al (1951).

I. PHYSICAL CHEMICAL STUDIES

Radioactive phosphorus was used in the study of physical chemical

factors. The following six factors were varied: pH, ionic strength, current strength, duration of iontophoresis, electrode size, and concentration. When any one factor was varied, the other five factors were maintained as constantly as possible. Also, as another physical factor, physical state was employed as another variable.

Voltage was measured by a Simpson voltmeter. The leads of the instrument were applied to the electrodes coming from the Golseth Fizzell Constant Current Generator and the voltage recorded. In all experiments, with the exception of current density variation, the voltage recorded was an approximately constant value.

1. pH MEASUREMENTS

The pH of the solution used at the "driving electrode" site was varied by the use of various buffer solutions. For a pH of 2.9 the KHPhthalate-KCl buffer was employed. At a pH of 7.1 a Na_2HPO_4-NaH_2PO_4 buffer was employed. Finally, at a pH of 10.1 a H_3BO_3-KCl-NaOH buffer was used. pH measurements of these different solutions was accomplished by a Beckman pH meter. The ionic strength in the three buffer solutions was approximately 0.1.

2. IONIC STRENGTH MEASUREMENTS

The ionic strength of the "driving electrode" fluid was varied by adding NaH_2PO_4 to the appropriate dilution of the radioactive solution. The ionic strength of the original diluted solution was assumed to be less than 0.001. Ionic strengths of 0.17, 1.02, and 2.00 were also used during this phase of the iontophoresis study.

3. CURRENT STRENGTH MEASUREMENTS

For this study, the iontophoresis current was applied at three strengths--seven and one-half milliamperes, five milliamperes, and two and one-half milliamperes. The other variable factors were controlled by using, in each case, the same dilution of stock radioactive isotope solution at the "driving electrode".

4. DURATION OF IONTOPHORESIS

Iontophoresis was applied for selected increments of time--15 minutes, 30 minutes, and one hour.

5. CONCENTRATION STRENGTH MEASUREMENTS

Various concentrations of the radioactive phosphorus were used during controlled iontophoresis. It was arranged so that an aliquot of the same dilution of the P^{32} solution was used for all concentration experiments. This was done by carrying out each experiment after an appropriate decay period. Thus the composition of P^{32} was altered without significantly changing the chemical composition and ionic strength of the solution. Four decrements of concentration were used---4,800,000, 1,020,000, 455,100, and 102,400 counts per minute.

In another experiment, attempts were made to introduce by iontophoresis three increments of concentration of strychnine sulfate--a 0.5 per cent solution, a 1.0 per cent solution, and a 2.0 per cent solution. Two criteria were used in comparing the physiological responses produced by the various concentrations of strychnine sulfate.

The latent period, the time from the beginning of the experiment until the initial appearance of typical generalized strychnine convulsions, and the survival or death of the animal after the experiment.

6. ELECTRODE SIZE MEASUREMENTS

Three different sizes of electrodes were employed to study the effect of this variable on iontophoresis. They were 4.69 cm^2, 6.25 cm^2, and 9.38 cm^2 at the driving electrode site. The conventional "driving electrode" used in all other experiments measured approximately 6.00 cm^2.

7. PHYSICAL STATE

Attempts were made to introduce by iontophoresis colloidal Au^{198}, I^{131} labelled albumen, and Insulin (Illetin-Lilly) so as to give us an index of the amount of these colloidal materials introduced into the rat as compared to the non-colloidal materials which we used previously.

In the case of Insulin, it was not tagged with any radioactive element. To determine the transfer of insulin, if any, we used as our criteria the fall in blood glucose values. Blood samples were taken from the rat by cardiac puncture before and after the experiment. Blood glucose was determined according to the method of Hoffman (1937).

II. INJECTION - IONTOPHORESIS

A comparison of the uptake by various tissues between iontophoresis, on the one hand, and intramuscular, subcutaneous, intraperitoneal, and intravenous injections on the other, was carried out with the use of P^{32}.

Approximately one to four microcuries were used with the injections as compared to forty microcuries with iontophoresis. An attempt was made to approximate the uptake pattern of tissue distribution of iontophoresis by injection techniques, using various concentrations of radioactive phosphorus solutions. We feel this would give us a rough estimate of the amount of material introduced into the body of the rat by iontophoresis. Also a comparison of the uptake pattern of radioactive phosphorus by various tissues of the rat, guinea pig, and rabbit was made, using approximately forty microcuries of P^{32}, applied at the "driving electrode" site, the anode.

With Ca^{45}, iontophoresis was compared to subcutaneous injection only. Approximately thirty-five microcuries of radioactive calcium were used in the subcutaneous injection as compared to 350 microcuries in iontophoresis.

A comparison of the uptake by various tissues using iontophoresis and subcutaneous injection was undertaken with the use of labelled diiodofluorescein, tagged with I^{131}. Approximately ten microcuries were administered by subcutaneous injection as compared to 100 microcuries by iontophoresis.

Approximately thirty-five and seventeen microcuries of Au^{198} were administered subcutaneously to the rat in a series of experiments, and the body distribution was measured by the procedures already mentioned. About 234 microcuries of colloidal Au^{198} were administered by iontophoresis.

III. TUMOR STUDIES

1. SOURCE

The tumor from which this strain arose was first found by Dr. George Walker of Baltimore, Maryland, in 1928 and was carried to

the present by successive transplantation in rats. The original source of the material which we used here was obtained from Dr. Frederick Benjamin, Department of Clinical Physiology, University of Illinois.

2. METHOD OF TUMOR TRANSPLANTING

Tumor transplants were made from an aseptic saline emulsion of finely crushed 10-12 day old tumor tissue, diluted up to a volume of 10 milliliters according to the method of Talalay et al (1952). Transplants were made in 0.2 milliliters doses injected subcutaneously into the inguinal region of the rat. It was noted that it was not until six or seven days after the transplant that the tumor became readily palpable. From the time it first made its appearance (approximating the size of a pea) the tumor grew with such rapidity that one week after first becoming palpable it approximated the size of a small lime.

3. IONTOPHORESIS WITH TUMORS

The size of the tumors used generally depended on the particular type of experiment that was to be performed. In most of our experiments on tumors we employed the use of two electrodes on opposite sides of the tumor growth. However, in a few other experiments we used only one electrode over the tumor growth and the other applied to an indifferent area of the body, usually over the chest region of the rat. The first category will be designated as "double electrode tumor experiments" and the latter as "single electrode tumor experiments".

Local distribution into the tumor as well as systemic distribution (obtained by taking various tissue samples of tissues from the rat) were determined after employing two iontophoresis procedures as described above, using P^{32} and Au^{198}.

(a) DOUBLE ELECTRODE TUMOR EXPERIMENTS

Approximately 234 microcuries of Au^{198} and approximately 35 microcuries of P^{32} were employed in iontophoresis with the Walker rat tumor. The tumors used were generally of large size, so that one could establish, by serial radioactivity measurements, the depth of penetration of the material used in iontophoresis in the various sections of the tumor. The tumor was divided up into three sections: (1) the area near the "driving electrode", (2) the area adjacent to the indifferent electrode, and (3) the middle area of the tumor. These three tissue samples were prepared for counting in the same fashion as we have already indicated for all tissue studies.

(b) SINGLE ELECTRODE TUMOR EXPERIMENTS

Experiments were performed with Au^{198} and P^{32} on small tumors where only the active electrode was placed over the tumor area. Approximately the same concentration of radioactivity was used as in the previous case where double electrodes were both placed on the tumor.

CHAPTER III

RESULTS

I. PHYSICAL CHEMICAL STUDIES

1. pH

In Figure 3 the per cent uptake of P^{32} per gram of tissue by the various tissues is indicated for the three ranges in pH--2.2, 6.8, and 10.0. In Table II these results are summarized. Approximately 4,642,000 counts per minute in two milliliters of solution were used at the appropriate driving electrode.

2. Ionic Strength

In Figure 4 the per cent uptake of P^{32} per gram of tissue by the various tissues is listed after four variations in ionic strength - 0.001, 0.17, 1.02 and 2.00. The results of this experiment are summarized in Table III. A concentration of 4,700,000 counts per minute in two milliliters of solution was applied at the appropriate driving electrode.

3. Current Density

In Figure 5 the per cent uptake of the P^{32} per gram of tissue by the various tissues and samples is indicated for the three increments of current strength that have been employed--2.5 milliamperes, 5 milliamperes, and 7.5 milliamperes. The results are summarized in Table IV. With this study the initial count was found to be 4,800,000 counts per minute for the two milliliters of solution as applied to the appropriate driving electrode.

4. Duration of Iontophoresis

In Figure 6 the per cent uptake of P^{32} per gram of tissue by the various tissues is indicated after three different time intervals of iontophoresis--60 minutes, 30 minutes, and 15 minutes. The results are tabulated in the same manner as with the studies involved in current density in Table V. The amount of radioactive phosphorus applied at the appropriate driving electrode was approximately 4,465,000 counts per minute in a volume of two milliliters of solution.

5. Electrode Size

In Figure 7 the per cent uptake of P^{32} per gram of tissue by the various tissues is indicated after varying the electrode size--4.69 cm^2, 6.25 cm^2, and 9.38 cm^2. These results are summarized in Table VI. Approximately 4,500,000 counts per minute were applied at the appropriate driving electrode.

6. Concentration

In Figures 8 and 9, the counts per minute uptake per gram of tissue of the P^{32} by the various tissues is indicated after four variations in concentration--4,800,000, 1,020,000, 455,100, and 102,400 counts per minute applied at the appropriate driving electrode. The results are summarized in Table VII.

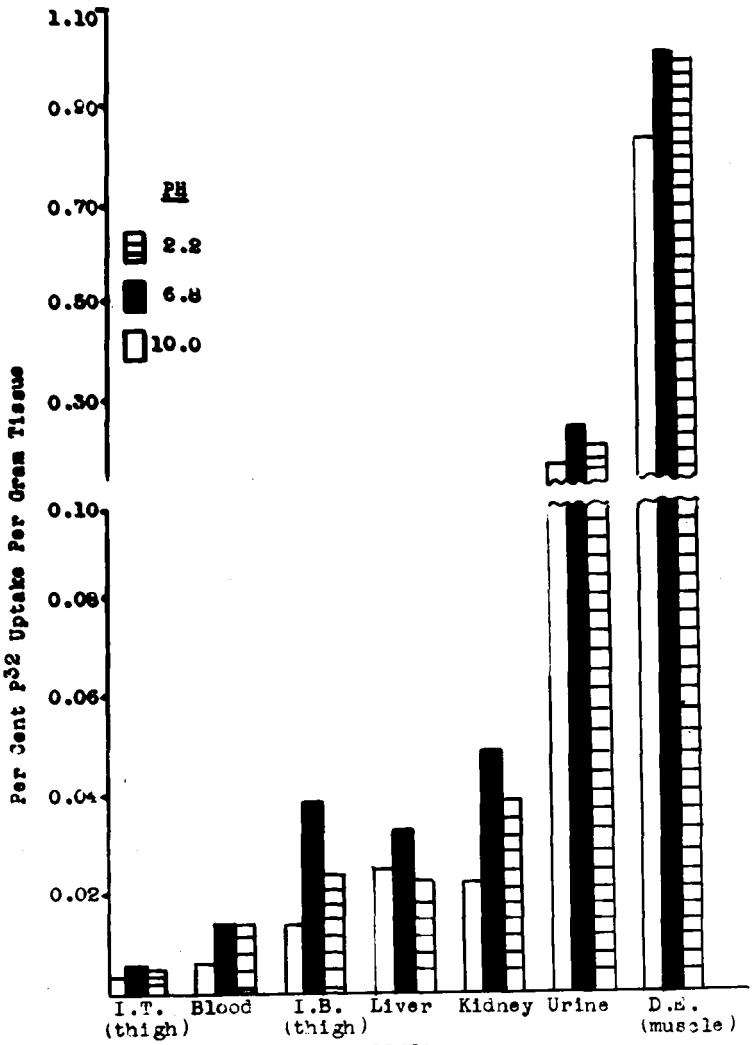
Table VIII lists the results of the experiments where strychnine sulfate iontophoresis was employed, using three variations in concentration--0.5%, 1.0% and 2.0%. Rats were the experimental animals used.

7. Physical State

In Figure 10 the per cent uptake of colloidal Au^{198} and I^{131} labelled

albumen per gram of tissue by the various tissues is indicated. Approximately 4,766,000 counts per minute of colloidal Au^{198} and 4,724,000 counts per minute of labelled albumen were applied at the appropriate driving electrode. These results are summarized in Table IX.

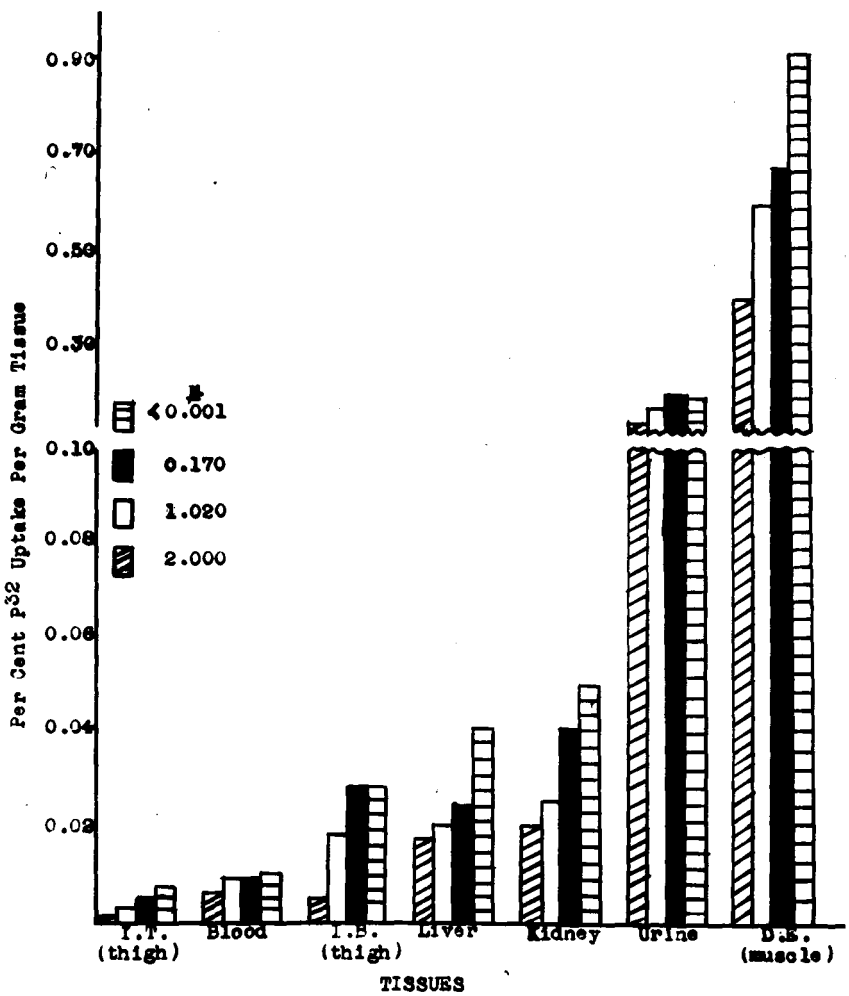
In another series of experiments, approximately 50 units of Insulin (Iletin-Lilly) were applied at the appropriate driving electrode. The results of the insulin iontophoresis experiments are listed in Table X.



Distribution of P^{32} With Variation in PH

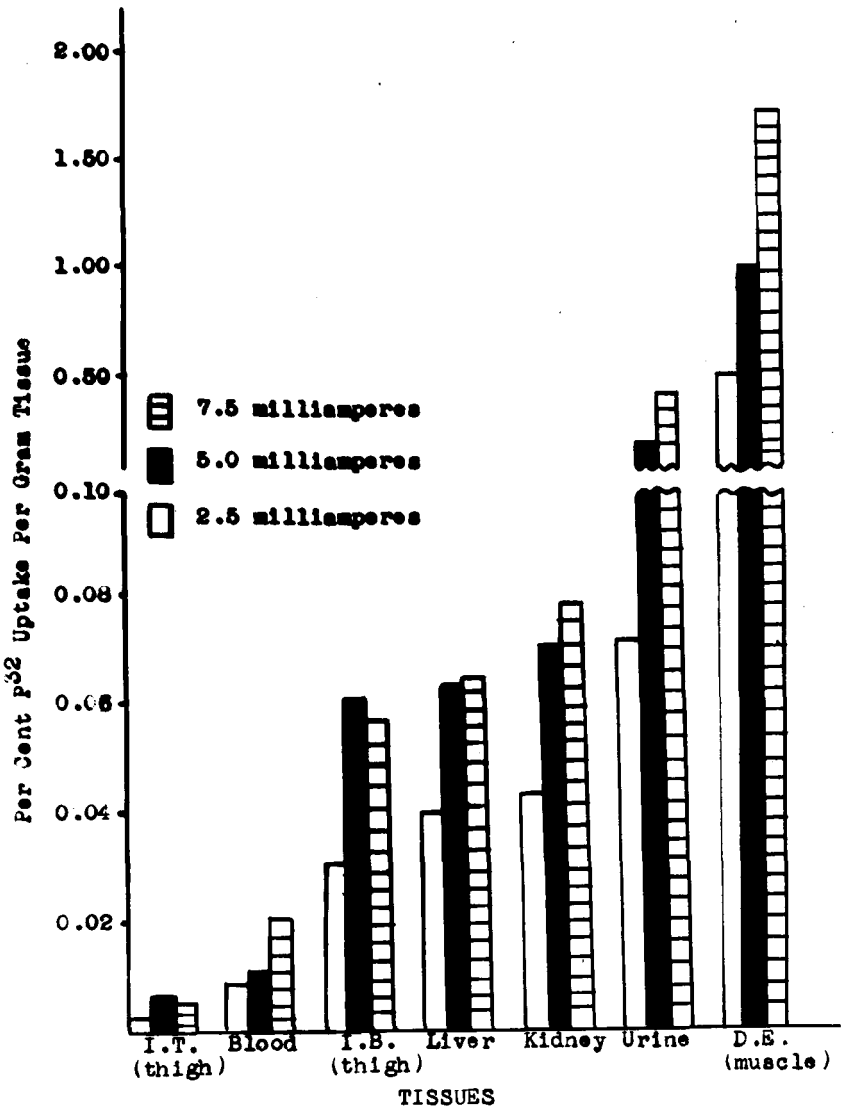
I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

FIGURE 3



Distribution of P^{32} With Variation in Ionic Strength
I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

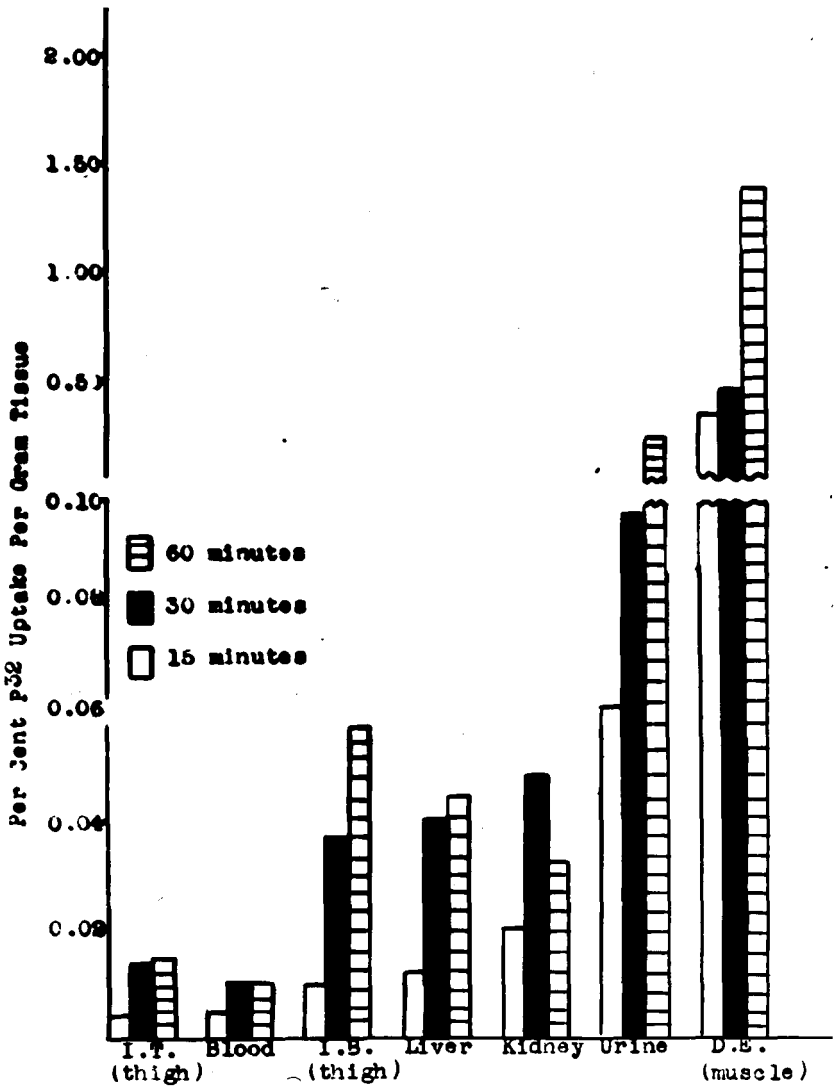
FIGURE 4



Distribution of P^{32} With Variation in Current Strength

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

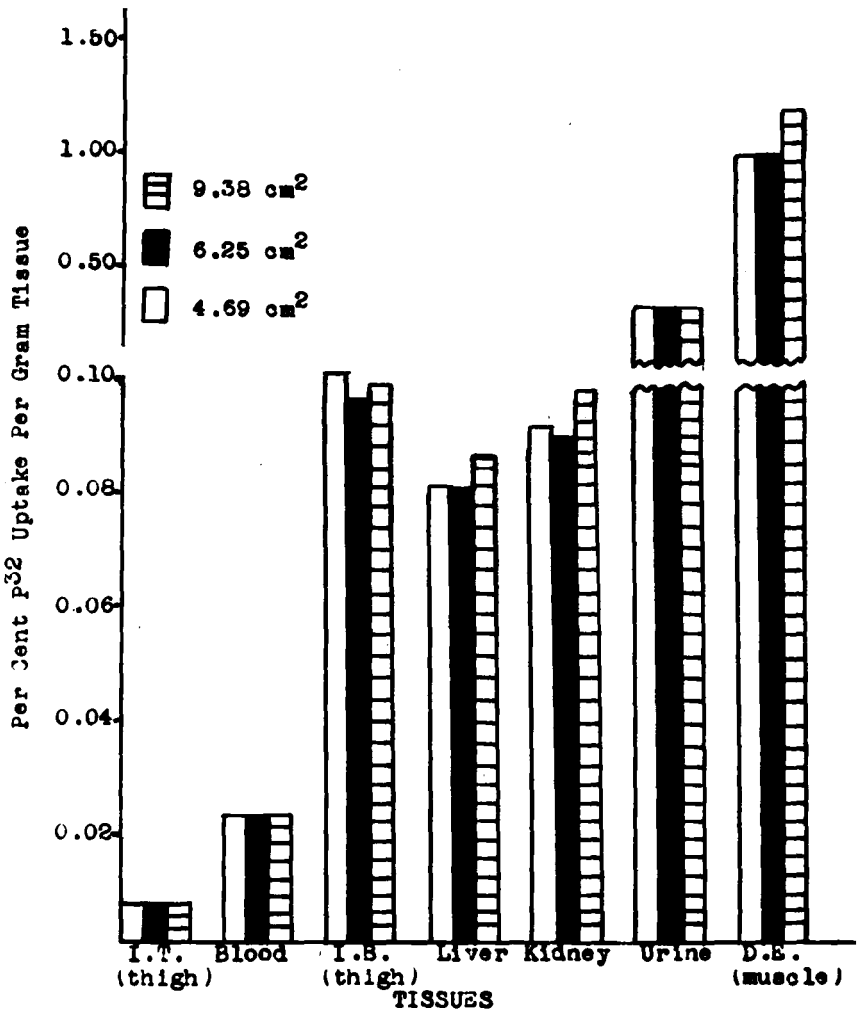
FIGURE 5



Distribution of P^{32} With Variation in Time

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

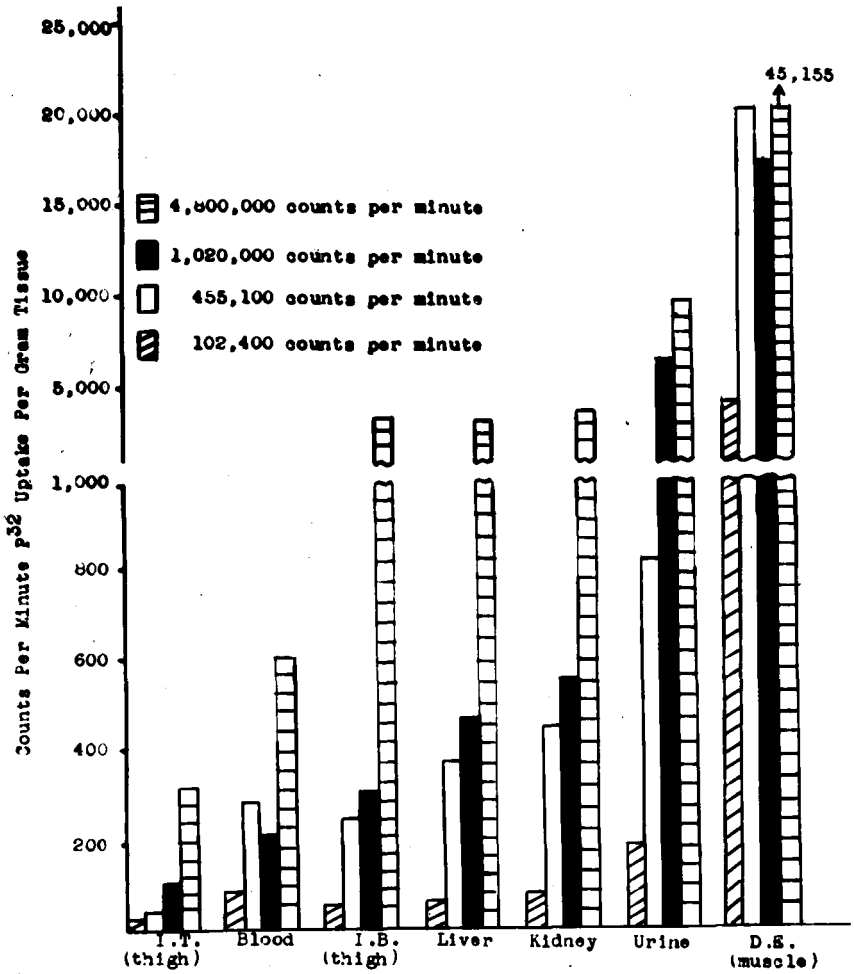
FIGURE 6



Distribution of P^{32} With Variation in Electrode Size

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

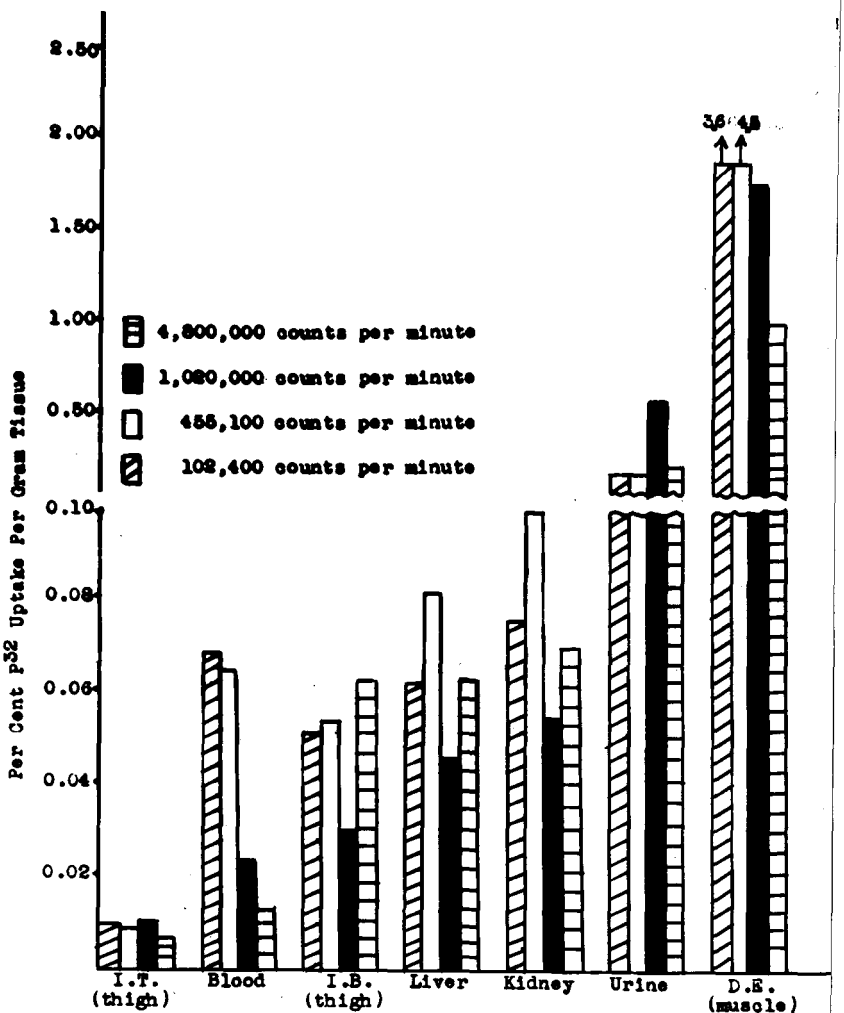
FIGURE 7



Distribution of P^{32} With Variation in Concentration
(Counts Per Minute)

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

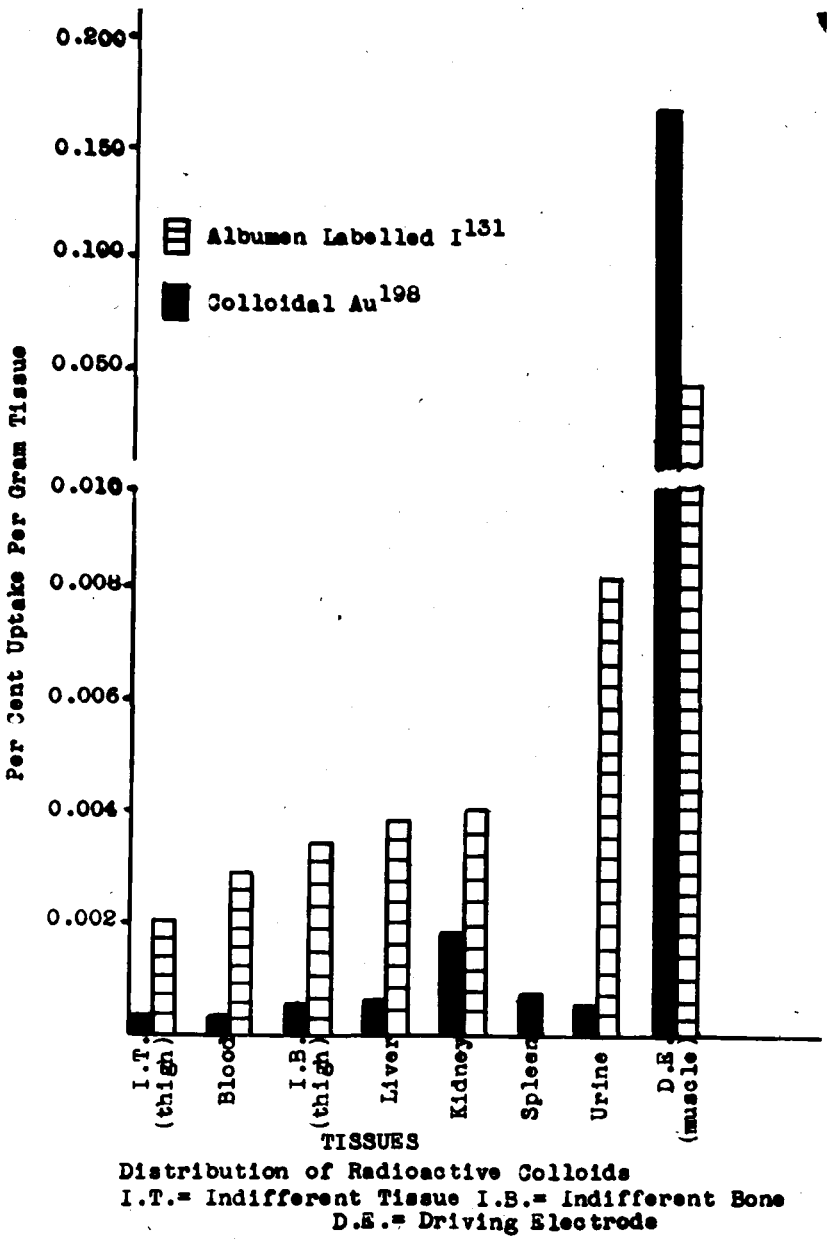
FIGURE 8



Distribution of P^{32} With Variation in Concentration (Per Cent Uptake)

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

FIGURE 9



Distribution of Radioactive Colloids
I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

FIGURE 10

TABLE II

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN pH
4,642,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT FOR ONE HOUR

TISSUES	pH 2.2		pH 6.8		pH 10.0	
	c/MIN. PER GRAM	% UPTAKE PER GRAM	c/MIN. PER GRAM	% UPTAKE PER GRAM	c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving Electrode	45,090	0.9713	47,787	1.0293	38,430	0.8278
Urine	10,607	0.2069	11,397	0.2456	7775	0.1674
Blood	587	0.0126	607	0.0130	257	0.0055
Liver	1030	0.0221	1497	0.0323	1137	0.0245
Kidney	1747	0.0376	2187	0.0471	1020	0.0219
Indifferent Muscle	227	0.0049	247	0.0053	177	0.0038
Indifferent Bone	1073	0.0231	1787	0.0384	627	0.0135

TABLE III

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN IONIC STRENGTH
4,700,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT FOR ONE HOUR

TISSUE	= 0.001		= 0.17		= 1.02		= 2.00	
	c/MIN. PER GM.	UPTAKE* PER GM.	c/MIN. PER GM.	UPTAKE* PER GM.	c/MIN. PER GM.	UPTAKE* PER GM.	c/MIN. PER GM.	UPTAKE* PER GM.
Driving Electrode	43,460	0.92	32,233	0.69	28,290	0.60	19,137	0.41
Urine	9382	0.20	9812	0.21	8335	0.18	6832	0.15
Blood	523	0.01	460	0.01	450	0.01	290	0.01
Liver	1940	0.04	1160	0.02	943	0.02	845	0.02
Kidney	2070	0.04	1900	0.04	1196	0.03	947	0.02
Indifferent Muscle	345	0.01	277	0.01	146	0.01	79	0.01
Indifferent Bone	1323	0.03	1273	0.03	840	0.02	273	0.01

* Per cent uptake per gram of tissue

TABLE IV

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN CURRENT DENSITY
4,800,000 c/MIN. APPLIED TO DRIVING ELECTRODE FOR ONE HOUR

TISSUES	7.5 MILLIAMPERES c/MIN. PER GRAM	7.5 MILLIAMPERES % UPTAKE PER GRAM	5 MILLIAMPERES c/MIN. PER GRAM	5 MILLIAMPERES % UPTAKE PER GRAM	2.5 MILLIAMPERES c/MIN. PER GRAM	2.5 MILLIAMPERES % UPTAKE PER GRAM
Driving Electrode	51,460	1.70	45,155	0.94	23,527	0.49
Urine	17,545	0.37	9,593	0.20	3562	0.07
Blood	980	0.02	603	0.01	401	0.01
Liver	3043	0.06	3020	0.06	1877	0.04
Kidney	3700	0.08	3335	0.07	2060	0.04
Indifferent Muscle	236	0.01	315	0.01	100	0.01
Indifferent Bone	2700	0.06	2951	0.06	1533	0.03

TABLE V

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN DURATION
4,465,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH

TISSUES	60 MINUTES		30 MINUTES		15 MINUTES	
	c/MIN. PER GRAM	% UPTAKE PER GRAM	c/MIN. PER GRAM	% UPTAKE PER GRAM	c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving Electrode	65,441	1.47	22,047	0.49	13,688	0.31
Urine	11,549	0.26	4318	0.10	2705	0.06
Blood	473	0.01	481	0.01	261	0.01
Liver	2007	0.04	1830	0.04	583	0.01
Kidney	1460	0.03	2163	0.05	913	0.02
Indifferent Tissue	690	0.02	687	0.01	213	0.01
Indifferent Bone	2580	0.06	1683	0.04	465	0.01

TABLE VI

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN ELECTRODE SIZE
4,500,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	9.38 cm^2 c/MIN. PER GRAM	9.38 cm^2 % UPTAKE PER GRAM	6.25 cm^2 c/MIN. PER GRAM	6.25 cm^2 % UPTAKE PER GRAM	4.69 cm^2 c/MIN. PER GRAM	4.69 cm^2 % UPTAKE PER GRAM
Driving Electrode	53,087	1.18	44,760	0.99	44,043	0.98
Urine	11,832	0.26	11,907	0.26	12,748	0.28
Blood	1063	0.02	1007	0.02	1000	0.02
Liver	3857	0.09	3570	0.08	3640	0.08
Kidney	4368	0.10	3987	0.09	4113	0.09
Indifferent Muscle	337	0.01	340	0.01	353	0.01
Indifferent Bone	4383	0.10	4283	0.09	4537	0.10

TABLE VII

TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN CONCENTRATION OF THE ISOTOPE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUE	4,800,000 c/MIN. c/MIN. PER GM.	% UPTAKE PER GM.	1,080,000 c/MIN. c/MIN. PER GM.	% UPTAKE PER GM.	455,100 c/MIN. c/MIN. PER GM.	% UPTAKE PER GM.	102,400/MIN. c/MIN. PER GM.	% UPTAKE PER GM.
Driving Electrode	45,155	0.94	17,640	1.73	20,517	4.51	3747	3.66
Urine	9,593	0.20	5440	0.53	816	0.18	184	0.18
Blood	603	0.01	215	0.02	293	0.06	70	0.07
Liver	3020	0.06	460	0.05	373	0.08	63	0.06
Kidney	3335	0.07	550	0.05	453	0.10	77	0.07
Indifferent Tissue	315	0.01	105	0.01	37	0.01	10	0.01
Indifferent Bone	2951	0.06	305	0.03	240	0.05	52	0.05

TABLE VIII

IONTOPHORESIS WITH STRYCHNINE SULFATE
VARIATION IN CONCENTRATION
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

ANIMALS (Rats)	CONCENTRATION	RESULTS
4	2.0%	4 animals died Onset of convulsions in 14 minutes
4	1.0%	1 animal died 3 animals survived Onset of convulsions in 17 minutes
4	0.5%	4 animals survived Onset of convulsions in 27 minutes

TABLE IX

IONTOPHORESIS WITH RADIOACTIVE COLLOIDS
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUE	COLLOIDAL Au^{198} 4,766,000 c/MIN. c/MIN. PER GRAM	% UPTAKE PER GRAM *	LABELLED ALBUMEN 4,724,600 c/MIN. c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving Electrode	7990	0.1676	2110	.0447
Urine	17	0.0004	387	.0082
Blood	13	0.0002	133	.0029
Liver	32	0.0007	177	.0038
Kidney	85	0.0018	193	.0040
Spleen	38	0.0008	-	-
Indifferent Muscle	10	0.0002	95	.0020
Indifferent Bone	25	0.0005	159	.0034

* Values less than 0.01 are considered not significant.

TABLE X

IONTOPHORESIS EXPERIMENTS WITH INSULIN
USING 60 UNITS - APPLIED AT THE DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

ANIMAL	BLOOD GLUCOSE BEFORE IONTOPHORESIS MILLIGRAMS PER CENT	BLOOD GLUCOSE AFTER IONTOPHORESIS MILLIGRAMS PER CENT
1	130	135
2	124	109
3	100	96

II. IONTOPHORESIS - INJECTION COMPARISON STUDIES

A comparison was made regarding tissue uptake with radioactive phosphorus in the rat by iontophoresis on the one hand, and on the other by subcutaneous, intramuscular, intraperitoneal, and intravenous injections.

With iontophoresis 4,026,000 counts per minute were applied to the driving electrode and the process carried out under routine conditions already discussed. Various tissue samples were taken and prepared for counting as mentioned previously, at the end of the experiment, which was one hour of duration in all of our studies.

Radioactive phosphorus was infiltrated subcutaneously around the front left leg in a series of rats in total concentrations of 341,330, 238,732, 204,700, 203,670, and 90,000 counts per minute. The resulting tissue distributions are listed in Table XI. Intramuscular injection using 208,269 counts per minute was employed also in the rat in the same area, but the material was infiltrated into the muscle. Intraperitoneal injection using 299,706 counts per minute of P^{32} and intravenous injection using 204,800 counts per minute of P^{32} was employed in a series of rats. In all of the injection studies the rats were sacrificed one hour after the administration of the radio-isotopes, and various tissue samples taken and digested as in the previous cases where P^{32} had been employed. A comparison of the uptake of P^{32} by the tissues from these various modes of administration of the radio-isotope is listed in Table XII and is illustrated in Figure 11.

Using I^{131} labelled diiodofluorescein, comparison studies were also

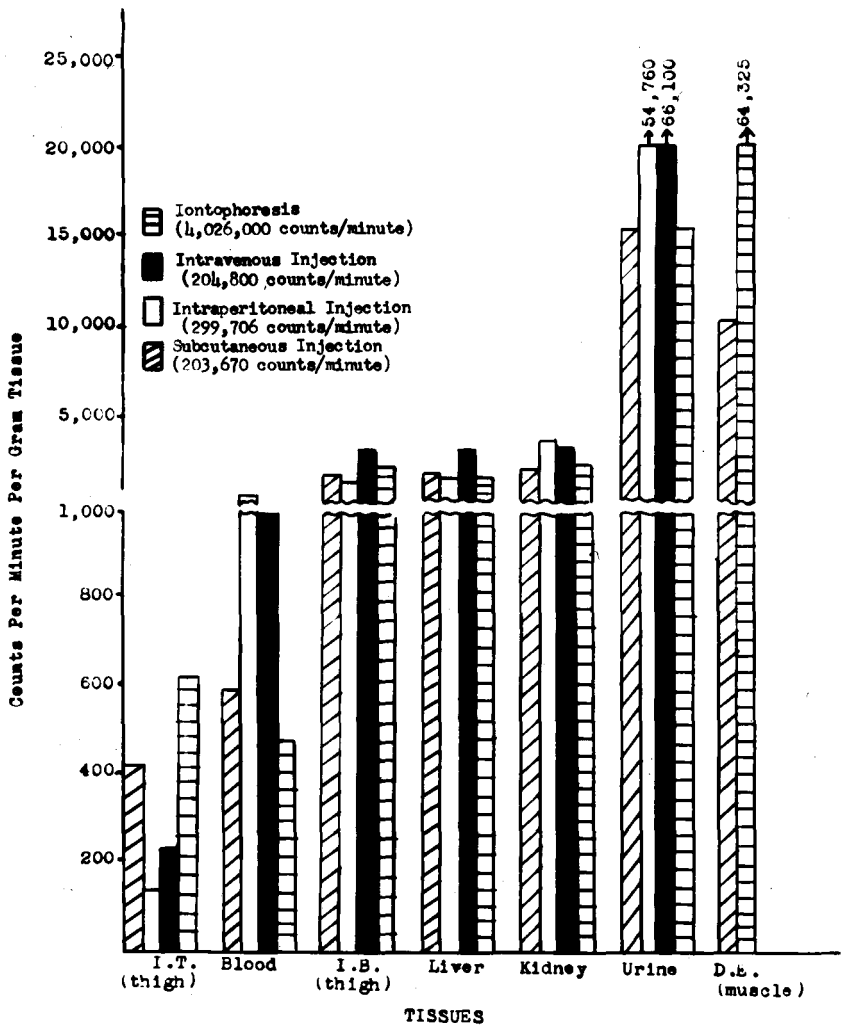
made between the counts per minute uptake by the tissues after iontophoresis with 3,060,000 counts per minute of radioactive material and subcutaneous injection of 304,200 counts per minute. After one hour the animals were sacrificed in both cases, and tissue samples taken and prepared for counting as has been discussed previously. The results of these experiments are listed in Table XIII, and are illustrated in Figure 12.

A comparison study regarding the counts per minute uptake by the tissues was made between iontophoresis and subcutaneous injection with Ca^{45}. In iontophoresis 1,060,000 counts per minute were used, and with subcutaneous injection 100,800 counts per minute were employed. The animal was sacrificed after one hour, the duration of the experiment in both instances, and tissue samples taken and prepared for counting in the manner that has already been discussed. The results of these experiments are summarized in Table XIII, and are illustrated in Figure 13.

A comparison in respect to the counts per minute uptake per gram of tissue by various tissues was made between iontophoresis and subcutaneous injection with colloidal radioactive gold. Iontophoresis was employed with approximately 4,766,000 counts per minute of Au^{198}, while subcutaneous injection was attempted with 819,200 counts per minute and 390,093 counts per minute, respectively. After one hour in each the rat was sacrificed, tissue samples taken and prepared as in the previous cases with radioactive gold. The results are listed in Table XIV.

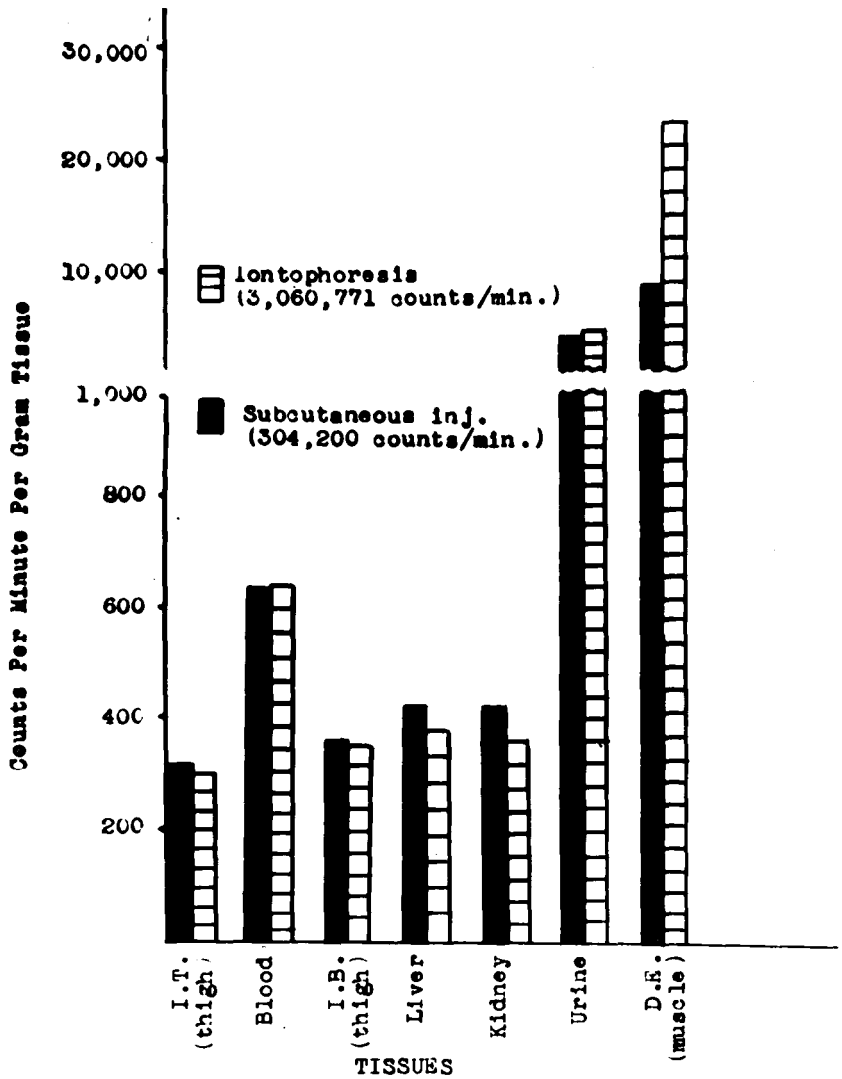
Finally, species variation studies using rats, rabbits and guinea pigs were employed, using iontophoresis with P^{32}. With rats 4,642,000 counts per minute were employed, with guinea pigs 4,214,000 counts per minute were used,

and for rabbits 4,048,000 counts per minute were utilized. Various tissue samples were taken and prepared for counting in the manner already described for P^{32}. In Table XV, the comparative results are summarized between iontophoresis in the rat, rabbit, and guinea pig. Also, in Figure 14, these results are illustrated.



Distribution of P^{32} Following Various Routes of Administration
I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

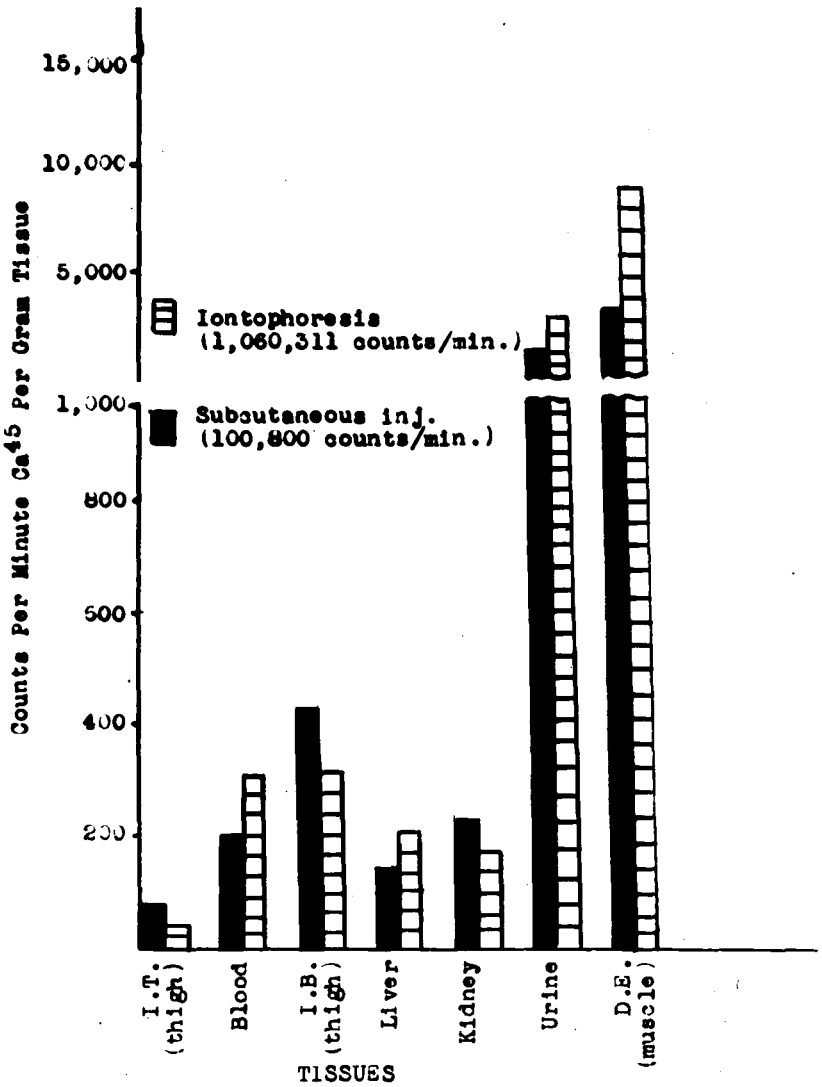
FIGURE 11



Distribution of I^{131} Labelled Diiodofluorescein After Iontophoresis And Subcutaneous Injection

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

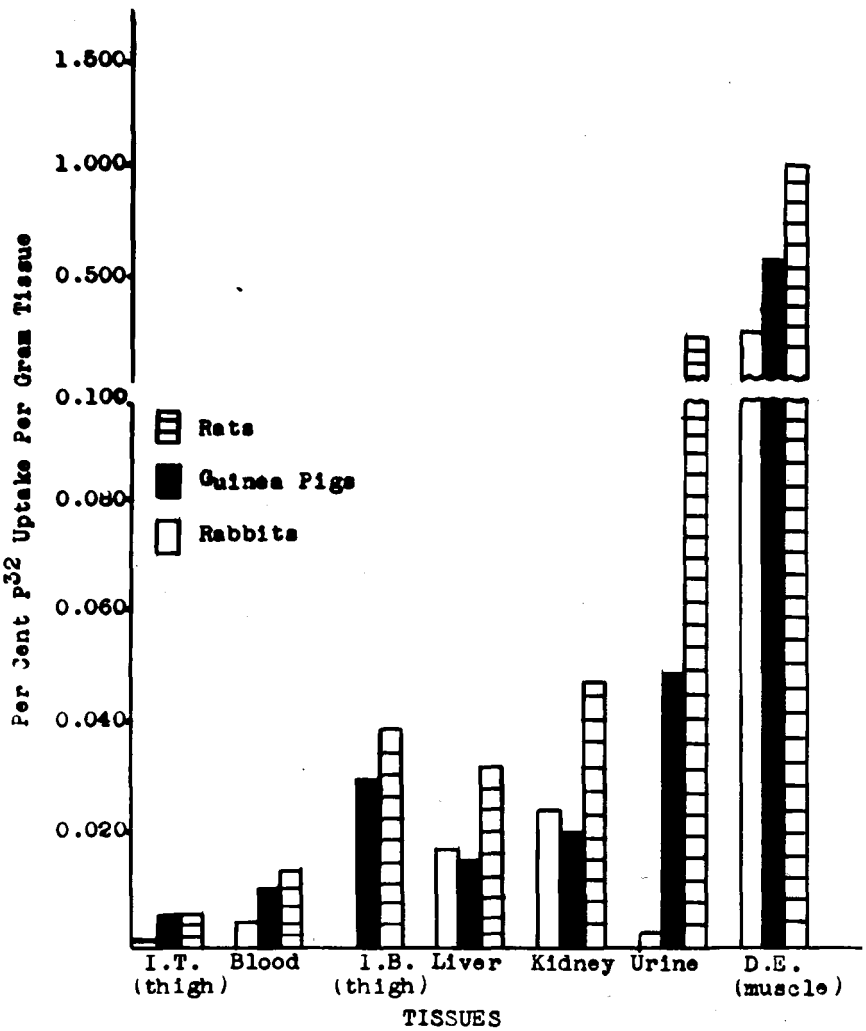
FIGURE 12



Distribution of Ca^{45} After Iontophoresis And Subcutaneous Injection

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

FIGURE 13



Distribution of P^{32} With Variation in Species

I.T.= Indifferent Tissue I.B.= Indifferent Bone
D.E.= Driving Electrode

FIGURE 14

TABLE XI

SUBCUTANEOUS INJECTION STUDIES
WITH
VARIOUS CONCENTRATIONS OF P^{32}

ALL SAMPLES TAKEN AFTER ONE HOUR

TISSUE	341,330 c/MIN. c/MIN. PER GRAM	238,732 c/MIN. c/MIN. PER GRAM	204,700 c/MIN. c/MIN. PER GRAM	203,670 c/MIN. c/MIN. PER GRAM	90,000 c/MIN. c/MIN. PER GRAM
Driving Electrode	18,000	14,730	10,700	10,319	8000
Urine	24,290	15,670	14.670	15,200	4568
Blood	1325	437	609	584	160
Liver	6211	2030	2160	2060	590
Kidney	5940	2760	2190	2150	640
Indifferent Muscle	490	450	620	420	150
Indifferent Bone	2230	2230	1920	1750	620

TABLE XXI

COMPARISON OF DIFFERENT ROUTES OF ADMINISTRATION OF P^{32}
ALL SAMPLES TAKEN AFTER ONE HOUR - FOR IONTOPHORESIS
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUE	IONTO-PHORESIS 4,026,000 c/MIN. APPLIED c/MIN. PER GRAM	SUB-CUTANEOUS INJ.-203,670 c/MIN. APPLIED c/MIN. PER GRAM	INTRA-MUSCULAR INJ.-208,269 c/MIN. APPLIED c/MIN. PER GRAM	INTRA-PERITONEAL INJ.-299,706 c/MIN. APPLIED c/MIN. PER GRAM	INTRA-VENOUS INJ.-204,800 c/MIN. APPLIED c/MIN. PER GRAM
Driving Electrode	64,325	10,319	10,940	-	-
Urine	15,200	15,200	14,770	54,760	66,100
Blood	472	584	533	1048	987
Liver	1980	2060	2380	1920	2573
Kidney	2230	2150	2320	3627	3263
Indifferent Muscle	620	420	310	137	233
Indifferent Bone	2400	1750	1160	1407	2763

TABLE XIII

COMPARISON OF IONTOPHORESIS WITH SUBCUTANEOUS INJECTION USING LABELLED DIIODOFLUORESCEIN AND Ca^{45} IN IONTOPHORESIS - FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

Tissue	Labelled Diiodo. Iontophoresis 3,060,000 c/Min. Applied c/Min. per Gram	Labelled Diiodo. Subcut. Inj. 304,200 c/Min. Applied c/Min. per Gram	Ca^{45} Iontophoresis 1,060,000 c/Min. Applied c/Min. per Gram	Ca^{45} Subcut. Inj. 100,800 c/Min. Applied c/Min. per Gram
Driving Electrode	23,540	8537	9240	3278
Urine	4550	3843	2900	1867
Blood	625	623	315	200
Liver	370	417	198	137
Kidney	360	410	170	230
Indifferent Muscle	295	310	40	77
Indifferent Bone	345	353	303	423

TABLE XIV

COMPARISON OF IONTOPHORESIS WITH SUBCUTANEOUS INJECTION USING COLLOIDAL Au^{198} IN IONTOPHORESIS - FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUE	IONTOPHORESIS (ANODE) 4,766,000 c/MIN. c/MIN. PER GRAM TISSUE	SUBCUTANEOUS INJECTION 81,920 c/MIN. c/MIN. PER GRAM TISSUE	SUBCUTANEOUS INJECTION 39,093 c/MIN. c/MIN. PER GRAM TISSUE
Driving Electrode	7990	8180	4940
Urine	17	464	70
Blood	13	140	40
Liver	32	770	230
Kidney	85	1990	1480
Spleen	38	280	120
Indifferent Muscle	10	110	60
Indifferent Bone	25	150	80

TABLE XV

SPECIES VARIATION USING IONTOPHORESIS WITH P^{32}
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	RAT 4,642,000 c/MIN. c/MIN. PER GRAM	RAT % UPTAKE PER GRAM	GUINEA PIG 4,214,000 c/MIN. c/MIN. PER GRAM	GUINEA PIG % UPTAKE PER GRAM	RABBIT 4,096,000 c/MIN. c/MIN. PER GRAM	RABBIT % UPTAKE PER GRAM
Driving Electrode	47,787	1.03	24,965	0.59	10,375	0.25
Urine	11,397	0.25	2060	0.05	45	0.01
Blood	607	0.01	430	0.01	173	0.01
Liver	1497	0.03	655	0.02	730	0.02
Kidney	2187	0.05	880	0.02	1010	0.02
Indifferent Muscle	247	0.01	220	0.01	18	0.01
Indifferent Bone	1787	0.04	1240	0.03	-	-

III. TUMOR STUDIES WITH IONTOPHORESIS

1. Single Electrode Tumor Experiments

Approximately 2,834,000 counts per minute of radioactive phosphorus were applied to the driving electrode over the tumor with the indifferent electrode placed over the chest area. Various tissues, including the tumor tissue, were taken and prepared for counting as already described. These experiments were compared to others in which the same type of electrode (cup shaped planchet) was employed, but in animals where there were no tumors present. The driving electrode was placed over the chest area and the indifferent electrode was placed over the lower abdominal region. Similar concentrations were used at the driving electrode—approximately 2,320,000 counts per minute. After iontophoresis the tissues were taken and prepared for counting in the same manner as previously. These results are summarized in Table XVI.

Approximately 4,986,000 counts per minute of radioactive colloidal gold was used over the driving electrode in the tumor area and iontophoresis maintained for one hour. The tumor was taken and prepared for counting in the same way as discussed previously for colloidal Au^{198}. Control experiments were also performed in which the cup shaped planchet electrodes were employed, but in animals where there were no tumors present. These results are tabulated in Table XVII.

2. Double Electrode Tumor Experiments

Approximately 3,457,000 counts per minute of P^{32} were applied to the driving electrode at one side of the tumor, using the cup shaped electrodes. After the experiments, one hour in duration, the various sections of the tumor

were taken and prepared for counting as has been mentioned previously for radioactive phosphorus. The resulting uptake by various sections of the tumor tissue is indicated in Table XVIII.

Colloidal Au^{198} with an approximate concentration of 4,766,000 counts per minute was applied to the driving electrode. The experiment was carried out for one hour, tissue samples taken and prepared for counting as mentioned for the radioactive gold previously. The resulting uptake by various sections of the tumor are summarized in Table XIX.

TABLE XVI

IONTOPHORESIS IN TUMOR AND NON-TUMOR RATS USING P^{32}
APPLIED AT THE DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	NON-TUMOR ANIMALS 2,824,000 c/MIN. APPLIED		TISSUES	TUMOR ANIMALS 2,320,000 c/MIN.	
	c/MIN. PER GRAM	% UPTAKE PER GRAM		c/MIN. PER GRAM	% UPTAKE PER GRAM
Driving Electrode (Muscle)	13,150	0.47	Driving Electrode (Tumor)	10,490	0.45
Urine	9810	0.35	Urine	9890	0.44
Blood	500	0.02	Blood	490	0.02
Liver	1480	0.05	Liver	1400	0.06
Kidney	1840	0.01	Kidney	1990	0.09

TABLE XVII

IONTOPHORESIS IN TUMOR AND NON-TUMOR RATS USING Au^{198}
EMPLOYING CUP SHAPED ELECTRODES
4,986,000 c/MIN. APPLIED AT THE DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	NON-TUMOR ANIMALS c/MIN. PER GRAM	TISSUES	TUMOR ANIMALS c/MIN. PER GRAM
Driving Electrode (Muscle)	210	Driving Electrode (Tumor)	308
Urine	Background	Urine	Background
Blood	30	Blood	Background
Liver	Background	Liver	Background
Kidney	Background	Kidney	Background

TABLE XVIII

IONTOPHORESIS ACROSS TUMORS USING P^{32}
3,457,000 c/MIN. APPLIED AT THE DRIVING ELECTRODE FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

	ANIMAL #1 c/MIN. PER GRAM	ANIMAL #2 c/MIN. PER GRAM	ANIMAL #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Tumor Area Adjacent to Driving Electrode	9781	9200	8550	9177
Middle Tumor Area	866	807	860	844
Tumor Area Adjacent to Indifferent Electrode	879	860	730	805

TABLE XIX

IONTOPHORESIS ACROSS TUMORS
USING Au^{198}
4,986,000 c/MIN. APPLIED AT THE DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

	ANIMAL #1 c/MIN. PER GRAM	ANIMAL #2 c/MIN. PER GRAM	ANIMAL #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Tumor Area Adjacent to Driving Electrode	398	261	218	292
Middle Tumor Area	134	169	126	143
Tumor Area Adjacent to Indifferent Electrode	93	154	122	123

CHAPTER IV

DISCUSSION AND CONCLUSION

In our experiments change in pH of the fluid at the driving electrode produced little significant effect on the uptake of radioactive phosphorus by various tissues. However, there was a slightly lowered penetration with a pH of 10. This could be due to some precipitation of the phosphate containing P^{32}. The preparation used was likely to precipitate in the higher pH ranges, above 7. We can conclude that under the conditions of our experiment pH did not appear to have a significant influence on the transport of P^{32} by the iontophoretic process. In the case of the protein solutions that we employed at the driving electrode for iontophoresis purposes, we used a pH which was far removed from the isoelectric point of that protein. This was done to insure that we would not get a so-called zwitterion effect, leaving the protein as a whole, electrical neutral. In this state, theoretically, the protein substance would not move across a conducting medium under the influence of electrical current.

The ionic strength of a strong electrolyte can be defined as the sum of the concentrations of each ion (in moles) multiplied by the square of their valences and divided by two. It is divided by two if both positive and negative ions are involved. In this series of experiments the concentration of P^{32} was kept constant, and the ionic strength of the non-radioactive buffer

increased. This can be explained by considering that as the ionic strength of the buffer is increased, the interionic attraction increases between the buffer and the ion we wish to introduce. Since the ion atmosphere has a charge opposite to the ion it will exert a retarding effect ("drag effect") upon the motion of the ion that we wish to introduce. This is the basis of the Debye-Huckel Theory on the ionization of electrolytes.

The experiments dealing with current density demonstrated that with an increase of current strength there was a greater introduction of the radioactive phosphorus in the various tissues. This can be explained partly on the basis of Faraday's Law, which in essence states that the amount of material deposited at either electrode is proportional to the quantity of electricity which passes through the system. Therefore, one can visualize that with a greater current flow the amount of material which is deposited will be greater. The amount of material driven into the tissues would not necessarily conform exactly to the amount expected under Faraday's Law. In addition to the electrical cell reaction, which Faraday's Law embraces, there is also a vital membrane phenomena operating when tissues are involved. However, it appears on the basis of our experiments that the amount of material deposited in the tissues of the rat by iontophoresis is roughly proportional to the quantity of electricity that we applied, although this is not a direct linear relationship.

When the time of iontophoresis was varied there was a relationship found between the length of time of iontophoresis and the total introduction of the radioactive phosphorus into the various tissues of the rat. The longer the duration in time the comparatively greater was the uptake. Since Faraday's Law is a function of time as well as current strength, varying the time of

iontophoresis would theoretically change the quantity of electricity which passes through a system. We note, however, there was little significant difference between the liver, kidney, indifferent muscle, and blood in the one hour and one-half hour samples. It could be that the quantity of radioactive phosphorus reaches its peak in these tissues in the one-half hour samples, and that further iontophoresis results in its being turned over by biological mechanisms about as fast as it was introduced by iontophoresis.

With variations in the size of the electrode, it was found that there was no apparent influence by this factor on the introduction of the radioactive material under the conditions of this experiment. This might be expected, as the voltage was approximately constant during the course of the experiment. With larger size electrodes, using constant current, it might be possible to get slight variations in the uptake of a given material.

It was found in our experiments that the concentration of P^{32} was decreased there was less overall turnover of this material in the various tissues, as far as counts per minute were concerned. According to Faraday's Law this is an unexpected result, if you considered only an electrical cell reaction and only P^{32}. In essence Faraday proved that the total amount of material transported at either electrode was independent of concentration but dependent solely on the quantity of electricity employed. However, with our studies using iontophoresis we are dealing with a membrane phenomena in addition to the Faraday Law effect. In addition, other non-radioactive ions were present. The total ionic strength of the solution used at the driving electrode was the same in each experiment. It would be well to point out the extremely minute amounts of radioactive phosphorus, in terms of weight, which

were used at the driving electrode in iontophoresis. Fifty microcuries of P^{32} are approximately equivalent to a weight of 1.7×10^{-4} micrograms of phosphorus. Therefore it can be seen that as one decreased further this amount of radioactive phosphorus, relative to the other ions present, there is less possibility that radio-phosphorus would be made available for penetration into the tissues. It was also noted that the per cent uptake per gram of tissue of the administered dose by the tissues was generally greater with the more dilute solution of radioactive phosphorus than the more concentrated. However, we wish to emphasize that in our experience the total amount of radioactive phosphorus moving into the tissue is somewhat proportional to the concentration used, although there is not a direct linear relationship. Similarly iontophoresis experiments with three different concentrations of strychnine sulfate demonstrated that the response to strychnine was proportional to its concentration insofar as lethal results were concerned.

With a change in physical state of the active materials, that is, from substances in solution to colloids, using I^{131} labelled albumen and Au^{198}, it was found that the amount of these materials introduced into the body of the rat by iontophoresis was considerably less than when non-colloidal materials were employed. Results after iontophoresis with colloidal Au^{198} indicated a greater amount of radioactive material introduced into the local tissue in comparison to the amount introduced using I^{131} labelled albumen. However, the body distribution of the I^{131} labelled albumen after iontophoresis appeared to be greater. This finding may be explained on the basis that colloidal Au^{198} is of smaller particle size than that of I^{131} labelled albumen. The size of

colloidal Au^{198} was in the order of 0.005 microns while that of I^{131} labelled albumen is in the order of 0.0275 microns. In terms of mobilization of a particular material from the local living tissue by vital processes, it is a known fact that colloidal Au^{198} is considered relatively immobile in that regard (probably due to the fact that it is a substance foreign to the body of the rat). Labelled albumen appears to be turned over by the vital processes in the tissues of the rat to a greater extent. In the case where insulin was used apparently no extensive penetration into the tissues of the rat occurred after iontophoresis, as the blood glucose values were not altered significantly. Since insulin, in molecular size, is of the order of magnitude of 0.005 microns, we might expect its quantitative penetration into the local tissues of the rat to be comparable to that of colloidal Au^{198}. They both presumably approach the same order of magnitude insofar as particle size is concerned. Such ideas regarding size prompted Clark (1919) to hypothesize that the quantity of ions of different drugs migrating in a given length of time varies approximately according to their "anatomic" weights.

In the experiments where P^{32} was administered by subcutaneous injection in varying concentrations it was found that the uptake by the body tissues of this radioactive material was proportional to the amount injected. The greater the concentration of the material injected, the greater was the availability of the radioactive material at the local site, and the greater the amount picked up by the capillaries and carried in the general circulation.

When various routes of administration such as subcutaneous, intramuscular, intravenous, and intraperitoneal injections were compared to iontophoresis the following results were observed. In general, iontophoresis can

be compared to subcutaneous or intramuscular injection. When approximately six to ten per cent of the amount of the P^{32} used in iontophoresis was administered by the subcutaneous or intramuscular route, it was found that a similar distribution in the various body tissues had ensued as with iontophoresis, with the exception of the driving electrode area. The tissue area immediately next to the driving electrode showed a much greater diffused distribution of radioactive phosphorus after iontophoresis than was seen after subcutaneous or intramuscular injection. This result has been postulated as a rational basis for the administration of various therapeutic agents by iontophoresis. To our knowledge there has been no detailed quantitative comparison prior to this work with radioactive isotopes. With intravenous and intraperitoneal injection there is roughly the same pattern of uptake as with the other parenteral administrations (intramuscular and subcutaneous injections) but greater amounts appear in various tissues much more rapidly. This more or less follows from our knowledge of drug actions--that various types of parenteral administration should be classified in the following order as far as rapidity of action is concerned: intravenous injection - intraperitoneal injection - intramuscular injection - subcutaneous injection - iontophoresis.

With other radioactive isotopes, such as Ca^{45} and I^{131} labelled diiodofluorescein, similar results were shown regarding the tissue distribution pattern when the material was administered by iontophoresis and compared to subcutaneous injection. The amount of radilactive material present at the local tissue area after iontophoresis was compared to that present after subcutaneous injection. The same conclusion applies to these radio-isotopes as we have mentioned previously for radioactive phosphorus.

A quantitative difference in tissue distribution following iontophoresis was found, using three species of animals--rat, guinea pig, and rabbit. It appears that the general pattern of distribution in tissues in the three species are similar; however, the relative quantities present in similar organs are different. This result is more or less expected, and was probably due to the different sizes of the animals, the guinea pig and the rabbit both being considerably larger than the rat. Therefore, under constant conditions, the amount of material entering by iontophoresis would be distributed over a larger area of the body in the case of the guinea pig and rabbit. This confirms the work of Strehl et al (1950) who demonstrated the amount of radioactive material (introduced by iontophoresis) found in the blood diminished according to the animal used: rat, guinea pig, and rabbit, in that order.

We employed radioactive colloidal gold (Au^{198}) in iontophoresis and subcutaneous injection studies. It was observed that after iontophoresis the Au^{198} remained primarily in the local driving electrode region, very little going to the other tissues. Similarly, with subcutaneous injection there was very little transport of the colloidal radioactive gold outside of the local tissue where it was injected. This experimentally confirms the reports of other research workers regarding the relative immobility of colloidal Au^{198} after injection. The quantity of colloidal Au^{198} getting into the tissues from the electrode site is very small when compared to the amounts of other radioactive isotopes that have been so introduced. This is very likely related to the colloidal nature of the radioactive gold preparation. Others have demonstrated by experiment that certain animal membranes or parchments block

the diffusion of colloids through it. This is likely to be the case here. We must conclude that the ability of colloidal radioactive gold to penetrate into the tissues was severely restricted under the conditions of our experiment.

In the tumor experiments on rats we used cup shaped electrodes instead of the conventional arrangement we had employed previously. The overall distribution outside of the local area by various tissues of the rat, using the cup shaped electrode experiments, was very similar to that which had been obtained using the conventional electrodes with both P^{32} and colloidal Au^{198}. The local deposition of material, after iontophoresis, was similar both as far as normal tissue and tumor tissue was concerned. When radioactive phosphorus (P^{32}) was used in such studies, we found a diminished concentration at the local driving electrode area in comparison to the same local site, when the conventional electrodes were employed.

Widespread systemic distribution could possibly prove disadvantageous in the attempt to irradiate an isolated tumor growth. Subsequent possible systemic damage due to irradiation of the body might result. Colloidal radioactive gold (Au^{198}) would not of necessity suffer from this handicap as it appears to be easily localized at a particular site in the body, usually at the point of introduction. In its use for possible tumor therapy, however, as indicated in our experiments with colloidal Au^{198} where iontophoresis was tried with two electrodes attached to the opposite sides of a large tumor, it was shown that insignificant amounts of radioactive material were transported throughout the tumor mass. In therapeutic practice about 21 millicuries of colloidal Au^{198}, by injection, is employed with tumors of 5 cc. in size.

Under the conditions of our experiments with iontophoresis we are getting, at best, a diffusion of approximately 4.4×10^{-13} microcuries of Au^{198} throughout the tumor. However, we believe that by iontophoresis, the particular material probably enters the actual cells of the tissues themselves. Therefore it might take much less actual material, introduced by iontophoresis, to produce the same effect as when that material is injected. Even considering this point, it seems very unlikely that beneficial effects would take place in this case, since there is such a tremendous difference between the amount used in injection and that which can be introduced into the tissues after iontophoresis. Therefore, as used in this study, it appears that it would be impractical to utilize iontophoresis as a method of introduction of radioactive materials in the therapy of tumors.

CHAPTER V

SUMMARY

1) It was demonstrated that under the conditions of our experiments, using iontophoresis with P^{32} in the rat, the distribution in the tissues of the material was proportional to the current density, duration of iontophoresis, and concentration of the radioactive phosphorus used. With strychnine sulfate it was found that pharmacodynamic effects, convulsions and death, were also proportional to the concentration of strychnine used at the driving electrode site. It was found as the ionic strength of the solution used in iontophoresis with radioactive phosphorus was increased there was a decrease in the radioactivity uptake by the tissues. The findings with colloidal Au^{198}, insulin, and albumen labelled with I^{131} indicate that these colloids are not readily transported into tissues by iontophoresis. Whether this is due to particle size or some other property common to colloids is not determined by these experiments. Finally, there appeared to be no significant effect of pH and electrode size on the P^{32} distribution in the animal after iontophoresis under the conditions of our experiments.

2) When iontophoresis and injection comparison studies were made in the rat, it was found that intramuscular and subcutaneous injection most nearly approximated the distribution pattern produced by iontophoresis. This was demonstrated with P^{32}, Ca^{45}, and I^{131} labelled

diiodofluorescein. When intraperitoneal and intravenous injections were made, using the same concentrations of radioactive phosphorus as were used in subcutaneous and intramuscular injection, it was found that a similar distribution pattern prevailed with all methods of administration, one hour after the administration, but with the exception that the tissues appeared to contain greater amounts of radioactivity after intravenous and intraperitoneal injection.

In all cases with the radioactive materials used, there was a greater deposition of the material at the local site after iontophoresis than there was in the comparable site after injection techniques.

Species variation seemed to have an influence on the total uptake of radioactive phosphorus after iontophoresis, although the distribution pattern was similar in each. Rats, guinea pigs, and rabbits showed decreasing amounts of radioactivity present per gram of their tissues, in that order.

3) Iontophoresis experiments with colloidal Au^{198} showed a small local deposition of the material with very small and insignificant amounts being present in other tissues. With subcutaneous injection again there appeared to be little turnover of the colloidal radioactive gold outside of the local tissue where it was injected. The results were unfavorable in the endeavor to introduce colloidal Au^{198}. The total amount of colloidal Au^{198}, as determined by counts per minute per gram of tissue, in the local area, was very small.

Colloidal Au^{198} did not appear to be diffused readily throughout

the tumor of the rat after iontophoresis. After iontophoresis with radioactive phosphorus there was a much greater diffusion of radioactivity throughout the rat tumor mass as compared to similar experiments using colloidal Au^{198}.

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APPENDIX

TABLE XX

PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT WITH VARIATION IN pH - 4,642,000 c/MIN. APPLIED TO DRIVING ELECTRODE FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

pH = 2.0

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	43,930	41,990	49,350	45,090
Urine	11,760	9,464	10,578	10,607
Blood	670	550	540	587
Liver	950	1050	1090	1030
Kidney	2090	1440	1710	1747
Indifferent Muscle	150	310	220	227
Indifferent Bone	1190	840	1190	1073

pH = 6.8

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	46,860	47,660	48,840	47,787
Urine	13,420	10,023	10,748	11,397
Blood	640	630	550	607
Liver	1170	1720	1600	1497
Kidney	2850	1870	1840	2187
Indifferent Muscle	120	240	380	247
Indifferent Bone	1790	1630	1940	1787

TABLE XX (CONTINUED)

pH - 10.0

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	39, 880	37,110	38,300	38,430
Urine	8, 040	6825	8460	7775
Blood	310	200	260	257
Liver	550	1880	980	1137
Kidney	1, 000	1000	1060	1020
Indifferent Muscle	90	250	190	177
Indifferent Bone	270	810	800	627

TABLE XXI

PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT WITH VARIATION IN IONIC STRENGTH - 4,700,000 c/MIN. APPLIED TO DRIVING ELECTRODE FIVE MILLIAMPERES OF CURRENT FOR ONE HOUR

$\mu = < 0.001$

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	41,740	45,320	43,320	43,460
Urine	10,023	8104	10,020	9382
Blood	500	520	550	523
Liver	2020	1840	1960	1940
Kidney	2040	1880	2290	2070
Indifferent Muscle	330	340	370	345
Indifferent Bone	1420	1070	1480	1323

$\mu = 0.17$

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	30,030	33,880	32,790	32,233
Urine	8056	11,190	10,190	9812
Blood	440	450	490	460
Liver	1010	1170	1300	1160
Kidney	1810	2040	1850	1900
Indifferent Muscle	270	290	270	277
Indifferent Bone	1290	1250	1280	1273

TABLE XXI (CONTINUED)

μ = 1.02

TISSUE	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	28,110	30,850	25,930	28,290
Urine	8073	8477	8456	8335
Blood	460	450	440	450
Liver	960	940	960	943
Kidney	1170	1220	1200	1196
Indifferent Muscle	120	150	170	146
Indifferent Bone	850	800	870	840

μ = 2.00

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	18,730	15,910	22,770	19,137
Urine	6725	7410	6360	6832
Blood	300	330	240	290
Liver	1010	820	704	845
Kidney	1070	980	790	947
Indifferent Muscle	60	79	97	79
Indifferent Bone	250	310	260	273

TABLE XXXI
PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN CURRENT DENSITY
4,800,000 c/MIN. APPLIED TO DRIVING ELECTRODE FOR ONE HOUR

7.5 MILLIAMPERES

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	51,260	54,950	48,170	51,460
Urine	18,125	18,337	16,172	17,545
Blood	950	1010	980	980
Liver	3290	3120	2720	3043
Kidney	4090	3750	3260	3700
Indifferent Muscle	170	220	269	236
Indifferent Bone	2390	3020	2690	2700

5.0 MILLIAMPERES

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	45,300	45,530	44,635	45,155
Urine	7806	13,650	7323	9593
Blood	640	650	519	603
Liver	3550	3420	2090	3020
Kidney	3930	3700	2375	3335
Indifferent Muscle	490	190	265	315
Indifferent Bone	3090	2740	3023	2951

TABLE XXII (CONTINUED)

2.5 MILLIAMPERES

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	27,430	20,670	22,480	23,527
Urine	2040	4940	3705	3562
Blood	330	573	300	401
Liver	1847	1964	1820	1877
Kidney	2130	1840	2210	2060
Indifferent Muscle	90	120	90	100
Indifferent Bone	1840	1379	1580	1533

TABLE XXXII
PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN DURATION
4,465,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH

60 MINUTES

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	68,711	61,020	66,592	65,441
Urine	11,936	11,221	11,890	11,549
Blood	574	390	455	473
Liver	1804	2190	2027	2007
Kidney	1180	1640	1560	1460
Indifferent Muscle	630	720	720	690
Indifferent Bone	2580	2420	3005	2580

30 MINUTES

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	28,711	20,890	16,540	22,047
Urine	3085	5040	4830	4318
Blood	515	451	476	481
Liver	2050	1570	1870	1830
Kidney	2340	1790	2360	2163
Indifferent Muscle	620	1020	330	657
Indifferent Bone	1200	2520	1330	1683

TABLE XXIII (CONTINUED)

15 MINUTES

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	EXPERIMENT #4 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	15,840	8860	15,930	14,122	13,688
Urine	3230	1190	3140	3260	2705
Blood	190	504	200	150	261
Liver	260	1400	330	340	583
Kidney	850	1980	480	340	913
Indifferent Muscle	120	400	170	160	213
Indifferent Bone	240	920	360	340	465

TABLE XIV

PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN ELECTRODE SIZE
4,500,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

9.38 cm^2

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	37,450	81,550	40,260	53,087
Urine	12,493	12,870	10,133	11,832
Blood	1070	1060	1060	1063
Liver	4090	3340	4140	3857
Kidney	4843	3670	4590	4368
Indifferent Muscle	370	320	320	337
Indifferent Bone	4910	4410	3830	4383

6.25 cm^2

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	42,850	45,400	46,030	44,760
Urine	10,130	12,230	13,360	11,907
Blood	1100	800	1120	1007
Liver	3250	3190	4270	3570
Kidney	3830	3530	4600	3987
Indifferent Muscle	290	410	320	340
Indifferent Bone	3670	4610	4570	4283

TABLE XIV (CONTINUED)

4.69 cm^2

TISSUE	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	40,690	45,860	45,580	44,043
Urine	16,140	13,500	8605	12,748
Blood	1080	830	1090	1000
Liver	3990	3400	3530	3640
Kidney	4290	4090	3960	4113
Indifferent Muscle	320	450	290	353
Indifferent Bone	5010	4750	3850	4537

TABLE XXV
PROTOCOL OF TISSUE DISTRIBUTION OF P^{32} IN THE RAT
WITH VARIATION IN CONCENTRATION OF THE ISOTOPE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

4,800,000 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	45,300	45,530	44,635	45,155
Urine	7806	13,650	7323	9593
Blood	640	650	519	603
Liver	3550	3420	2090	3020
Kidney	3930	3700	2375	3335
Indifferent Muscle	490	190	265	315
Indifferent Bone	3090	2740	3023	2951

1,020,000 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	18,160	24,215	10,545	17,640
Urine	3310	6570	6440	5440
Blood	230	210	205	205
Liver	390	480	510	460
Kidney	660	430	560	550
Indifferent Muscle	100	110	105	105
Indifferent Bone	510	150	250	305

TABLE XXV (CONTINUED)

455,100 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	19,560	22,030	19,960	20,517
Urine	715	946	786	816
Blood	300	300	280	293
Liver	360	390	370	373
Kidney	470	450	440	453
Indifferent Muscle	30	50	30	37
Indifferent Bone	280	190	250	240

102,400 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	3750	3390	4130	3747
Urine	122	246	185	184
Blood	60	20	130	70
Liver	64	21	105	63
Kidney	80	51	101	77
Indifferent Muscle	0	0	30	10
Indifferent Bone	59	29	65	52

TABLE XXVI

PROTOCOL OF TISSUE DISTRIBUTION IN THE RAT
AFTER IONTOPHORESIS WITH COLLOIDAL Au^{198}
4,766,000 c/MIN. APPLIED TO DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	9530	2100	12,340	7990
Urine	16	20	15	17
Blood	10	20	10	13
Liver	39	28	30	32
Kidney	86	83	85	85
Spleen	31	37	46	38
Indifferent Muscle	8	8	14	10
Indifferent Bone	22	20	33	25

TABLE XXVII

TISSUE DISTRIBUTION IN THE RAT
AFTER IONTOPHORESIS WITH I^{131} LABELLED ALBUMIN
4,724,000 c/MIN. APPLIED AT THE DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	2480	1730	2120	2110
Urine	660	186	314	387
Blood	140	150	110	133
Liver	150	190	190	177
Kidney	270	170	140	193
Indifferent Muscle	90	110	84	95
Indifferent Bone	150	165	152	159

TABLE XXVIII

TISSUE DISTRIBUTION IN THE RAT
AFTER IONTOPHORESIS WITH I^{131} LABELLED DIIODOFLUORESCEIN
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

3,060,000 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	24,511	22,570	23,540
Urine	4100	5010	4555
Blood	580	670	625
Liver	390	350	370
Kidney	380	320	350
Indifferent Muscle	330	260	295
Indifferent Bone	370	320	345

TABLE XXIX

TISSUE DISTRIBUTION IN THE RAT
AFTER INTRAMUSCULAR INJECTION OF I^{131} LABELLED DIIODOFLUORESCEIN
304,200 c/MIN.
ALL SAMPLES TAKEN AFTER ONE HOUR

INJECTION I.M. - 304,200c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	9010	8260	8340	8537
Urine	4000	3820	3710	3843
Blood	700	620	550	623
Liver	440	400	410	417
Kidney	440	420	370	410
Indifferent Muscle	310	300	320	310
Indifferent Bone	380	350	330	353

TABLE XXI

TISSUE DISTRIBUTION IN THE RAT
AFTER IONTOPHORESIS WITH Ca^{45}
1,060,000 c/MIN. APPLIED AT DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

	1,060,000 c/MIN.		
TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	8560	9920	9240
Urine	2800	3000	2900
Blood	350	280	315
Liver	116	280	198
Kidney	180	160	170
Indifferent Muscle	50	30	40
Indifferent Bone	315	290	303

TABLE XXXI

TISSUE DISTRIBUTION IN THE RAT
AFTER INTRAMUSCULAR INJECTION OF Ca^{45}
WITH 100,800 c/MIN.
ALL SAMPLES TAKEN AFTER ONE HOUR

I.M. INJECTION - 100,800 c/MIN.

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	3954	3120	2760	3278
Urine	2000	1780	1820	1867
Blood	138	211	250	200
Liver	160	130	120	137
Kidney	280	220	190	230
Indifferent Muscle	90	70	70	77
Indifferent Bone	430	410	430	423

TABLE XXXII

TISSUE DISTRIBUTION IN THE RAT
AFTER INTRAPERITONEAL INJECTION OF P^{32} - WITH 299,706 c/MIN.
ALL SAMPLES TAKEN AFTER ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Urine	55,090	58,250	50,940	54,760
Blood	1257	1253	634	1048
Liver	1450	2430	1880	1920
Kidney	3930	3240	3710	3627
Indifferent Muscle	130	160	120	137
Indifferent Bone	1010	1620	1590	1407

TABLE XXXIII

TISSUE DISTRIBUTION IN THE RAT
AFTER INTRAVENOUS INJECTION OF P^{32} - WITH 204,800 c/MIN.
ALL SAMPLES TAKEN AFTER ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	EXPERIMENT #3 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Urine	62,580	69,000	66,720	66,100
Blood	1320	650	990	987
Liver	2580	2290	2850	2573
Kidney	3610	2890	3290	3263
Indifferent Muscle	390	120	190	233
Indifferent Bone	2670	2750	2870	2763

TABLE XXXIV

TISSUE DISTRIBUTION IN THE GUINEA PIG
AFTER IONTOPHORESIS WITH P^{32}
4,214,000 c/MIN. APPLIED AT DRIVING ELECTRODE
FIVE MILLIAMPERES FOR ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	25,280	24,650	24,965
Urine	1940	2180	2060
Blood	410	450	430
Liver	620	690	655
Kidney	860	900	880
Indifferent Muscle	220	200	220
Indifferent Bone	1150	1300	1240

TABLE XXXV

TISSUE DISTRIBUTION IN THE RABBIT
AFTER IONTOPHORESIS WITH P^{32}
4,096,000 c/MIN. APPLIED AT DRIVING ELECTRODE
FIVE MILLIAMPERES OF CURRENT STRENGTH FOR ONE HOUR

TISSUES	EXPERIMENT #1 c/MIN. PER GRAM	EXPERIMENT #2 c/MIN. PER GRAM	SUMMARY c/MIN. PER GRAM
Driving Electrode	10,710	10,040	10,375
Urine	70	20	45
Blood	160	185	173
Liver	720	740	730
Kidney	1030	990	1010
Indifferent Muscle	10	26	18
Indifferent Bone	-	-	-

APPROVAL SHEET

The dissertation submitted by Edward P. O'Malley has been read and approved by five members of the faculty of the Stritch School of Medicine, Loyola University.

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

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

20 May 54
Date

W. T. Foster
Signature of Adviser