ABSORPTION OF DRUGS FROM SUBCUTANEOUS CONNECTIVE TISSUE

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I. INTRODUCTION

As far as the present reviewer is aware, the subcutaneous absorption of drugs has not been reviewed in this century. Several reviews on gastrointestinal absorption and capillary permeability in general, however, have continued to appear. The explanation is the physiological importance of the last-mentioned subjects, whereas subcutaneous absorption is of interest mainly to pharmacologists in connection with administration of drugs.

The task has been both simple and complicated: simple because the literature has not been reviewed recently; complicated because of the sparse number of experimental articles devoted primarily to subcutaneous absorption. The information appears in papers and in abstracts under varying topics such as connective tissue, capillary permeability, capillary blood flow, absorption, *etc.* Therefore, valuable papers may have been overlooked by the reviewer. The authors of such papers will be first to understand the difficulties in finding the literature about the subject, and will probably be the first to excuse the reviewer. Furthermore, many of the studies concerning subcutaneous absorption have been performed in the research departments of the drug industry in attempts to meet the practical requests for techniques for delaying the action of injected drugs; such studies have often remained unpublished.

In an attempt to make the review clear and concentrated, only problems related specifically to subcutaneous absorption have been considered. We have refrained from considering subjects of more general interest, such as absorption and passage through capillary membranes. Although the physicochemical properties and lipid solubility of the drugs are important factors for the rate of absorption, they will be mentioned only in passing. Attention has been concentrated on how treatment with drugs and hormones, administered locally or systemically, may alter the rate of absorption by influencing the condition of the connective tissue, the capillary membranes, or the capillary flow. These limitations will stress our defective knowledge about the problems specific to subcutaneous absorption. The first recognition of the absorptive effect of subcutaneously injected drugs will be mentioned as an introduction. It is hoped that there will be a renaissance of the theoretical study of the mechanism of subcutaneous absorption in coming years.

II. EARLY HISTORY OF SUBCUTANEOUS INJECTION

Injection of drugs into the subcutaneous connective tissue is a relatively new method of drug-administration. Injection of blood and drug solutions intravenously has been known and used for more than three hundred years. The first subcutaneous injections described in the literature, however, were performed only about one hundred years ago.

The late introduction of the subcutaneous administration of drugs is explained by the fact that reasonably accurate syringes with piston and graduation scale, intended for small volumes of fluid, were first produced in the first half of the twentieth century. These were constructed for the injection of solutions of ferric chloride into cutaneous telangiectasias; an example is the syringe constructed by Pravaz (104) in 1853. This syringe has incorrectly been connected with the history of subcutaneous injection.

The first subcutaneous injection mentioned in the literature was administered by Alexander Wood of Edinburgh in 1853 (146). He used a syringe constructed by Ferguson for his injections of morphine in cases of neuralgia, where he intended to obtain a local analgetic action on the pains. Extensive information about the early history of the injection syringes and subcutaneous injections is given in historical reviews by Howard-Jones (63) and Mogey (96). Therefore only a few points of special interest that have not been stressed before will be mentioned here.

Undoubtedly Alexander Wood has the honour of being the first in the literature to describe the method of subcutaneous injection. However, it is also certain that Wood believed in only a local action of the injected drugs, as shown by the title of his first publication: *New Method of Treating Neuralgia by the Direct Application of Opiates to the Painful Points.* He stressed that the injections were to be given at the point of maximal tenderness to palpation, rather than where the patient localized the pains.

Usually the London surgeon Charles Hunter is given the honour of having realized first the systemic action of subcutaneously injected drugs. In 1858 he

wrote (67) that he had ". . . given up the localization of the remedy as proposed by Dr. Wood. . . ." He started to inject the drugs at different non-diseased parts of the subcutaneous tissue and found: ". . . by the injection of the narcotic into the cellular tissue of a part distant from that affected with the neuralgia, the relief that follows appears quite as great as when the injection is into the cellular tissue of the neuralgia part." Thus, Charles Hunter stressed that the effect of a drug injected subcutaneously was independent of the topographic localization of the injection zone in relation to the diseased tissue.

The same point of view, however, had been propounded five months earlier by Benjamin Bell (9) who advised the use of subcutaneous injections of opiates or belladonna in the treatment of unconscious patients intoxicated with these two drugs. Bell writes that in these patients: ". . . absorption from the enfeebled stomach . . . may . . . not be counted on: we possess, in subcutaneous injections, a more direct, rapid, and trustworthy mode of conveying our remedy in the desired quantity into the circulating blood." In a case history Bell noted also that when he injected morphine to a patient suffering from sciatica ". . . into the glutaeal region of the opposite limb, which happened to lie next the edge of the bed," an absorptive effect of the subcutaneously injected drug occurred, and that the action was excellent.

Too little attention—or, more correctly, no attention—has been given to Benjamin Bell as the first to mention in print the post-absorptive action of subcutaneously injected drugs. The basis is apparently a question of human character. Charles Hunter understood the value of his own invention and knew how to secure its acceptance. In 1865 (68) he first mentioned his own *hypodermic method*, as applying to medicines thus injected for general effects in contrast to what he called *the local injection of Wood*. This gave rise to a prolonged controversy between the two doctors, which was concluded by a report from a committee appointed by the Medical and Chirurgical Society in London to resolve the matter. In this report (91) it was concluded that ". . . no difference has been observed in the effect of a drug subcutaneously injected, whether it be introduced near to, or at a distance from the part affected."

III. METHODS FOR STUDYING THE RATE OF ABSORPTION

Following a subcutaneous injection, a series of events occur simultaneously in the dynamic system. The drug passes from the subcutaneous depot to the blood (absorption), then from the blood to the tissues (distribution), and finally processes of elimination occur (excretion through the urine, sweat, bile, expired air, *etc.*, or chemical transformation). The true rate of absorption is defined as the amount of the injected substance that passes from the injected depot into the blood per unit of time. To examine the rate of absorption we should possess ideally a model consisting of an absorption zone and the vascularization of the area. To date this remains only an ideal. Therefore, many experimental methods have been introduced in attempts to develop methods for evaluating the rate of absorption in the dynamic system.

The first review on subcutaneous injections was given by Eulenburg in 1865

(43). In the third edition of his monograph, that appeared in 1875, the author mentioned the existing methods for the study of the rate of absorption, namely, 1) registration of the occurrence of systemic symptoms produced by the absorbed substance, 2) chemical analysis of the drug in the circulating blood, and 3) chemical determination of the drug in secretions or excretions of the injected organism. To date, only one more method can be added: 4) recording of the clearance of the injected drug from the local area. The last method is theoretically the method of choice. It gives a true picture of the amount absorbed, if we can exclude local spreading and inexact quantitative analysis of the remaining amount in the local area as sources of error. These factors are limitations of the method. Therefore, the first three methods mentioned are still in use to evaluate the rate of absorption, especially when the problem is the determination of action of drugs that influence the rate of absorption.

A. Recording of post-absorptive symptoms

The drawback of this method is evident when it is used to evaluate whether a given systemic treatment influences the rate of absorption from a subcutaneously given solution of a drug. An alteration of the time from injection of the latter until a certain symptom or sign occurs may be caused by a change in rate of absorption. Other possible explanations are qualitative or quantitative changes in the distribution of the drug, in the compartment available for distribution, in the mechanism of inactivation of the drug, in the sensitivity to the action of the drug, and in the mechanism of drug-elimination. When the problem is the elucidation of whether a substance added locally to the drug solution influences the absorption of the drug, the method is of more value. If the adjuvant substance is given in small amounts relative to the primary drug, a systemic action of the former may be excluded as the cause of the recorded alterations in the "absorption time." Therefore, the method has been used properly to demonstrate the delaying action of epinephrine on subcutaneous absorption (13), and the promotion of subcutaneous absorption produced by the addition of hyaluronidase to the injected drugs (4, 70, 71, 130).

B. Analyzing drugs quantitatively in circulating blood

If the problem is the evaluation of the effect on the rate of absorption of a substance given directly in the solution containing the test drug, then this method gives a direct answer. Examples of such substances are hyaluronidase (46, 81) and histamine (116, 117). As mentioned previously, the problems are more complex if the task is an attempt to evaluate the effect of systemic treatment on the absorption of drugs from subcutaneously injected depots. The rise of the initial part of the time-concentration curve cannot be taken directly as a measure of the rate of absorption, because it is also influenced by the factors already considered. The common view is that alterations in the initial part of the time-concentration curve are produced mainly by differences in the rate of absorption (141). However, if the volume of the compartment of distribution or the other factors mentioned are altered to a high degree, this may easily be recognized even

in the initial part of the curve. As a general rule, studies should be carried through as double cross-over experiments (115). To verify that an alteration demonstrated in the time-concentration curve determined on intact animals is caused by an alteration in absorption, the effect should be verified in doubly nephrectomized animals to exclude a renal mechanism of action (118). The concentration following intravenous infusion at logarithmically decreasing rates to intact animals should be unaltered by the treatment (121).

C. Chemical analysis in excretions and secretions

A thorough consideration of these methods will not be given, because of their limited practical value and use in studies concerning subcutaneous absorption. The information desired concerns the passage through the capillary membrane. In the dynamic system, the distance from the capillary membrane to the biological fluid on which analysis is performed is even larger, and too many indefinite factors are interposed to give valuable information. A similar method has been used to evaluate the permeability of the synovial membrane, when coloured substances were given intraarticularly and quantitative determinations are conducted on the urine; evidently, the method is unsatisfactory if only the urine is analyzed (11, 12, 40, 60, 101, 124, 125, 126, 127, 128, 129).

D. Tissue clearance methods

From a theoretical point of view, these methods are the most satisfactory. The drawback is that significant amounts of the injected substance may be removed from the local area of injection otherwise than by absorption through capillary membranes. The sources of error are 1) spreading from the local area and 2) drainage through the lymphatic system. The evaluation of the decrease in a subcutaneously injected depot may be recorded in two ways. The first allows only one analysis from each experimental animal; this involves excision of the local zone of injection, extraction of the injected substance from the tissue, and its quantitative analysis. The second method attempts to record the clearance of the injected drug from the surface of the injection site. Ideally, this method should allow a continuous registration of the remaining drug in the injected zone during the whole length of the absorptive phase.

With the first procedure, the aim is to extract the whole volume of tissue in which the drug may have spread. This should be done without extracting too large an amount of tissue, because of the potential error of obtaining large amounts of absorbed material from retained peripheral blood in the vascular system. Therefore, the animals should be bled before the sample of tissue is excised (6, 62). The addition to the injection of a coloured indicator has been employed in order to limit the excision to the tissue in which the drug has spread. An example is the addition of methylene blue to injected solutions of epinephrine (49). This may not be correct if the penetration of the two drugs through the tissue is unequal, which may be influenced by the experimental conditions. However, the greatest disadvantage of this method is that only a single sample

for analysis can be obtained from each animal. This precludes the possibility of performing cross-over experiments to elucidate the effect of a given treatment.

Therefore, the method of following the clearance of the injected substances from the surface of the site of injection appears to be more attractive. Although it is an intracutaneous method, the wheal-disappearance test should be mentioned here (84). This test consists in recording the time from injection until disappearance of the wheal. It is very similar to the quantitation of the spreading reaction which was developed five years later (37, 38, 39). Neither of these methods yields information about the rate of absorption of compounds dissolved in the injected fluid (8).

In recent years the method of choice for tissue clearance experiments has been the injection of solutions containing radioactive tracers. It may be considered a further development of the procedure of injecting coloured substances, the absorption of which was evaluated from the disappearance of the colour from the injected tissues (13).

The most commonly used tracer is $^{24}Na^+$ in $^{24}NaCl$. Kety claimed in 1948 (77) that the disappearance of $^{24}NaCl$ from solutions injected subcutaneously in the human leg might be taken as a measure of the conditions of the peripheral circulation. The disappearance rate was delayed by epinephrine and by an arterial tourniquet (78). Many authors have used $^{24}Na^+$ and $^{131}I^-$ for investigations of subcutaneous absorption (46, 47, 73, 85, 90, 138, 144). When the radioactivity is measured over the surface of the skin, variations in the filtering effect of the tissue (possibly caused by the treatment given) and the spreading of the injected fluid from the injected depot influence the results. The concentration should be determined also in the circulating blood as a control (46, 85). Still, the variations in the results obtained with tissue clearance experiments using radioactive tracers are large, and therefore the results need statistical evaluation. Although the method includes many advantages, we still do not possess an ideal method for the study of subcutaneous absorption, such as the model mentioned in the beginning of Section III.

IV. MECHANISM OF SUBCUTANEOUS ABSORPTION

A. Peripheral circulation

Three major questions must be considered regarding the importance of the peripheral circulation for the subcutaneous absorption of drugs: 1) Does the sole or major degree of absorption into the blood occur locally in the zone of injection? 2) Through which vessel walls do drugs pass from the tissue depot to the circulating blood? 3) Is the passage due only to diffusion, or do processes such as filtration or active transport influence the absorption of drugs from the perivascular tissue into the circulating blood?

Concerning the first question, it was shown as early as 1892, by Asher (2), that subcutaneously injected sodium iodide is absorbed directly into the blood stream, while absorption through the lymphatic system *via* the thoracic duct into the blood is of no quantitative significance. He found that sodium iodide was rapidly demonstrable in the blood when injected subcutaneously on the crus

of a rabbit in which the thigh was cut, leaving only the main artery and vein intact. Starling and Tubby (137) also showed experimentally direct hematogenous absorption, and invalidated the theory of Heidenhain according to which absorption is chiefly *via* the lymphatic system, and only secondarily into the blood. This has been verified by later workers, including Okuneff (97) and Wepf (145), using different methods. Following subcutaneous injection of isotonic solutions of sodium chloride into the legs, Stone and Miller (138) found that only 1% of the injected salt passed the thoracic duct, while the rest passed through the femoral vein. Thus, many investigations have confirmed direct absorption into the blood.

We have less knowledge from which to answer the second question. It has not been possible experimentally to elucidate whether molecules are absorbed only through the walls of capillaries, or whether small veins or other vessels also take part in the process of absorption.

This problem is closely related to the third question, namely, whether absorption is due only to diffusion of molecules through the vessel walls. If this is the case, absorption must occur predominantly through the capillary walls. This can be shown mathematically, primarily because of the much higher frequency of capillaries in the tissue, which results in a shorter mean distance of diffusion to capillaries than to veins. A second line of evidence is the structure and thinness of capillary walls in comparison with other vessels, and a third is the much larger total surface of capillaries, compared to other vessels, in a zone of peripheral tissue (141). The three factors mentioned all point to the capillaries as the only important vessels concerned with subcutaneous absorption.

In 1895 Starling (136) advanced his brilliant theory about the paracapillary circulation (*i.e.*, the exchange between the capillaries and the extracellular fluid), about which there is no doubt today. Starling thought that filtration occurs in the arterial end of capillaries, due to the predominance of hydrostatic pressure over the plasma colloid osmotic pressure, while reabsorption occurs in the venous part of the capillaries, where the colloid osmotic pressure exceeds the hydrostatic pressure in the vessels. Another explanation has been given by Chambers and Zweifach (19), who claimed that outward filtration occurs in specialized arteriovenous capillaries, in which the hydrostatic pressure is higher than in true capillaries because of shorter lengths and larger intraluminal diameters at the arterial end. In the true capillaries they postulated that only reabsorption (inward filtration) occurs.

As mentioned before, it is commonly believed that dissolved substances are absorbed by diffusion from the perivascular fluid into the blood through the capillary walls. Because of the paracapillary circulation, there must exist a fluid continuum through the capillary wall, possibly in pores through the interendothelial substance or the endothelial cells (98, 99, 105).

Hyman *et al.* (73) claimed that the paracapillary circulation has no influence on the exchange of crystalloids between the interstitial tissue and the circulating blood. They injected solutions containing $^{131}I^-$ and $^{24}Na^+$ subcutaneously into the hands of humans, and measured the radioactive clearance from the

surface of the skin. The addition of 12.5% human albumin to the injected solutions did not alter the absorption rate, as indicated by the lack of significant difference in the rate of tissue clearance. However, there may have been a difference that could not be demonstrated with the rather crude method of measuring radioactivity over the skin of the injection zone. Therefore, more significance may be attached to the results of Madison and Christian (90) who found that the absorption of subcutaneously injected $^{24}NaCl$ was delayed by the addition of high concentrations of glucose to the injected solution. These experiments were performed on rats, and absorption was evaluated by analyzing the concentration of $^{24}NaCl$ in blood sampled by cardiac puncture. Using this method, the variations from a varying filter effect of the tissue are eliminated; therefore, the results are more reliable (85).

In accordance with these results, a pronounced reduction in the absorption of subcutaneously injected sodium sulphacetamide was found when histamine was added to the injection solutions (116, 117). Histamine injected into the subcutaneous tissue produces edema, in the course of which outward filtration must exceed inward filtration. These experiments therefore indicate that a solvent drag (*i.e.*, a pronounced net flow of filtration) influences the absorption. Experimental evidence is given for the belief that filtration influences the absorption of drugs to some degree, when there is an inwardly or outwardly directed net flow for the paracapillary circulation. If inward and outward filtration are equivalent, however, the rate of the paracapillary circulation will be without influence on the absorption rate.

When the paracapillary circulation is in equilibrium, only diffusion will be of significance for absorption. We have no knowledge or experimental evidence that active transport influences subcutaneous absorption. The factors that determine the absorption rate are therefore the factors that influence diffusion. Among these the total area of the absorbing capillary membrane should be mentioned first. In addition, absorption is proportional to the diffusion coefficient, the gradient of concentration, and the distance of diffusion (thickness of the capillary membrane). If the vessels in the absorption zone remain unchanged, the absorption rate is enhanced when the flow of blood is increased, because the concentration of the absorbed drug is lowered in the blood of the absorbing capillaries, which leads to a higher concentration gradient.

The number of active capillaries in the absorption zone is of importance for the total absorbing surface. It should be stressed, however, that the total flow of blood through the absorption zone is not a direct measure of the capillary flow, and the capillary flow is the only thing of interest regarding absorption. The condition of the arteriovenous shunts must be taken into consideration, because they are able to shunt significant percentages of the total flow from the arteries to the veins (110) without passing the only vessels of significance for absorption, *i.e.*, the capillaries.

B. The absorbing membrane

The absorbing membrane in subcutaneous connective tissue is the capillary wall. A general quantitative description of drug absorption through this mem-

brane has not yet been given, at least not a description based on experimental data. Although qualitative and quantitative investigations concerning the exchange of substances between the blood and the interstitial fluid have appeared in recent years, experiments on capillaries in subcutaneous tissue have not been carried out specifically. However, from information obtained on the passage of substances through capillaries in certain specific tissues and through capillaries in general, assumption may be drawn concerning subcutaneous absorption.

Teorell in 1937 developed a mathematical theory to describe the kinetics of distribution of substances injected into the body (141, 142). He confined his investigations to cases where the electrical forces are weak, or to cases concerned with the administration of non-electrolytes. The amount of a drug that permeates a certain tissue boundary was assumed to depend on Fick's law for unidimensional molecular diffusion:

$$-dN = D \times \frac{dc}{dx} \times A \times dt, \tag{1}$$

where $-dN$ = amount, D = diffusion coefficient, dc/dx = concentration gradient, A = surface, dt = time. The equation for the rate of passage from a depot (for example, following subcutaneous injection) to the blood was given the form:

$$-\left(\frac{dN}{dt}\right)_{D-B} = k_1'\left(\frac{x}{V_1} - \frac{y}{V_2}\right), \tag{2}$$

where x = amount in depot, V_1 = volume of depot, y = amount in blood, V_2 = volume of blood. k_1' is a "permeability coefficient" in which is included the effective permeation surface, the boundary thickness, and the diffusion coefficient for the injected drug. With the assumption that $\frac{y}{V_2}$ can be neglected, equation 2 expresses the rate of absorption as proportional to the depot concentration:

$$-\left(\frac{dN}{dt}\right)_{D-B} = \frac{k_1'}{V_1} \times x = k_1 \times x. \tag{3}$$

As stressed by Kety (79) in his review on the theory of the exchange of inert gas at the lungs and tissues, Teorell did not include the capillary blood flow in his equation, and offered no explanation for this exclusion. The decrease in the rate of absorption caused by epinephrine and the demonstration that the tissue clearance of intramuscularly injected $^{24}NaCl$ is dependent on the perfusion of an extremity clearly demonstrate that capillary perfusion is a limiting factor for absorption in many circumstances (78). For the clearance process, Kety gave the following equation:

$$\frac{dQ}{dt} = -Q\left(\frac{mF_v}{s} + \frac{nF_L}{s}\right), \tag{4}$$

where Q = total content of $^{24}NaCl$ in the unit mass of tissue, F_v = venous outflow per minute, F_L = lymphatic outflow per minute. m and n are constants

between 0 and 1 expressing the extent to which capillary blood and lymph come to equilibrium with the tissue concentration of $^{24}NaCl$.

While it was assumed earlier that filtration was of major importance for the exchange of substances between blood and interstitial fluid, it now seems a fact that most substances pass the capillary membrane by diffusion. Thus, Kruhøffer showed in 1946 that inulin leaves the circulation more slowly than sucrose, the respective rates being approximately proportional to the aqueous diffusion coefficients of the two substances (80).

Pappenheimer *et al.* (99) developed a method for studying quantitatively the permeability of capillaries to lipid-insoluble molecules. Their results were obtained from experiments on perfused hindlegs of cats and dogs. This method was used earlier for evaluating the filtration permeability (100). The results from these and later investigations with the same and similar preparations (105, 106, 107) have recently been reviewed by Renkin and Pappenheimer (108). In these experiments most of the perfusion passes through muscle capillaries. However, part of the total perfusion passes capillaries in other tissues, *i.e.*, subcutaneous tissue, skin, and bones. The explanation given by these authors about the passage of substances through capillary walls will be summarized briefly.

The transcapillary diffusion coefficient for lipid-soluble agents is related to their oil-water partition coefficients. The diffusion rate of such substances is much faster per unit concentration difference than that of lipid-insoluble molecules of comparable size. Lipid-soluble molecules are believed to diffuse through all of the capillary wall, consisting of the endothelial cells. This type of permeability is comparable to the permeability of cell membranes in general. Renkin and Pappenheimer stated that lipid-insoluble molecules, as well as water and electrolytes, diffuse through pores penetrating the capillary wall, and that the surface available for diffusion of these compounds amounts to less than 0.1% of the total capillary wall. The quantitative data obtained on the isolated hindlimb may be explained by the assumption of 1 to 2×10^9 pores of a mean radius of about 30 Å per cm^2 capillary wall. Net volume flow through the pores is caused by hydrodynamic processes. However, filtration and absorption due to hydrodynamic flow is slow compared to plasma flow. The exchange of smaller molecules between blood and interstitial fluid is mainly due to diffusion.

The authors observed that the passage of even small molecules was not as rapid as would be expected from free diffusion. To explain the decrease in apparent pore area as a function of increasing molecular size, a concept of "restricted diffusion" was introduced. The restriction to diffusion is pronounced for larger lipid-insoluble molecules. Therefore a concentration gradient will exist through the capillary wall. In this case the passage of molecules through the membrane will be influenced to a high degree by filtration. With abnormally high rates of filtration, the passage of even such small molecules as urea and of Na and Cl ions may theoretically be influenced by filtration.

Chinard *et al.* have used another method for elucidating the mechanism of transcapillary exchange of water and soluble substances (20). Blood is sampled continuously from a vein from an organ or extremity immediately after in-

stantaneous injection of a test substance into the artery of the organ. A non-diffusible reference substance (commonly, Evans Blue, T-1824) is dissolved in the injection solution. This method allows measurement of the loss of a test substance simultaneously with the dilution of the substance in the blood. A similar method has been used by Freis *et al.* (48) and by Crone (24). The latter author attempted a quantitative description of the passage of non-electrolytes through the walls of capillaries in the brain. Chinard *et al.* concluded that the passage of water and of other substances across the capillary wall occurs by a mechanism of diffusion.

Qualitatively, the permeability of capillary walls in subcutaneous tissue hardly differs from that of muscular capillaries. It is therefore possible to draw an analogy from the above mentioned findings to subcutaneous absorption. Accordingly, the rate of absorption for lipid-soluble substances is believed to depend on their oil-water partition coefficients, whereas lipid-insoluble substances are absorbed at a rate depending on their diffusion rates in aqueous solution. The rate of filtration is believed to influence the rate of absorption to some degree. This should always apply to large lipid-insoluble molecules. A pronounced net flow of filtration, a solvent drag, would be expected to influence the absorption rate of most lipid-insoluble substances, even those of small molecular size.

C. Connective tissue ground substance

Subcutaneous injections are given into connective tissue. The condition of this "organ" influences to what extent injected solutions are exposed to capillary membranes for absorption. The connective tissue may be altered by systemic factors or by locally acting stimuli. This may influence the rate of subcutaneous absorption.

The injection of fluids into subcutaneous connective tissue requires a certain pressure, the injection pressure (92). The tissue exerts an elastic resistance to injection, as can be studied by infusion experiments. The resistance is suddenly reduced at a critical initial pressure, after which the infused volume per second increases proportionally to a rising injection pressure. The initial pressure at which infusion starts is about 30 to 40 mm water. It is comparable to the breaking point as demonstrated in dermal connective tissue (87, 88, 89).

Only one experimental investigation has been made to elucidate whether the pressure used when giving subcutaneous injections influences the rate of absorption. Barke (6) showed that the absorption of glucose is reduced when injected subcutaneously using injection pressures amounting to 1 to 2 atmospheres. The author explained the phenomenon as the result of traumatic ischaemia, without mentioning anything about possible haemorrhages into the tissue. This will be discussed later.

The cause of the elastic resistance to injection below a critical pressure is in the structure of the connective tissue. A theory about the construction of the subcutaneous connective tissue has been given by Day (35), which is based on a series of experiments (28, 29, 30, 31, 32, 33, 34). The connective tissue includes cells, fibrils, and interstitial substance. Day considered it constructed as a net-

work woven by fibrils, in the interstices of which a finer network is formed by long chains of protein molecules. Finally, the interstices are filled by hyaluronic acid, swollen in the interstitial fluid. This view may agree with investigations using electronic microscopy (50). The elastic resistance to injections may be produced by the swollen hyaluronic acid and the network of protein molecules. However, an explanation of the critical breaking point and the proportionality of infusion to pressure cannot be given.

The clear demonstration of the importance of connective tissue ground substance for absorption is given by the effect of hyaluronidase on the absorption rate. In 1947 it was shown that the addition of hyaluronidase to saline solutions infused subcutaneously under constant pressure increased the rate of infusion to a high degree (55, 56). This has been reproduced by many later workers in different areas of connective tissue (1, 17, 18, 25, 26, 51, 123). The most consistent action quantitatively is found when the pressure is measured under constant rate of infusion (81).

The increase in the rate of infusion with constant pressure could be due to local spreading, and does not directly demonstrate increased absorption. However, many investigators have shown that the absorption of drugs and other chemicals from injected solutions is increased by the action of hyaluronidase. Only a limited number of such investigations will be mentioned here (4, 46, 71, 130).

The final explanation of the absorption-promoting action of hyaluronidase is still unknown. It may be explained solely by the increased permeability and reduced hydrophilic capacity of the connective tissue, which causes the injected drug solutions to spread over a larger tissue area. Such an expansion of the zone of absorption includes a greater number of capillaries, and accordingly a larger total capillary membrane area takes part in the absorption, which consequently is accelerated. There are no signs to indicate a solvent drag directed against the capillaries during the action of hyaluronidase. When hyaluronidase in small volumes of fluid is injected into connective tissue, the content of water is unaltered. Therefore, the balance of the paracapillary circulation remains in equilibrium. Several authors have stated that there is a capillary permeability-increasing action of hyaluronidase. However, their experimental results may be explained by impurities in the hyaluronidase preparations, such as histamine or other permeability factors. A more thorough discussion of this problem will be given later. Another question is whether an increased permeability of capillaries, in the sense of permeability to larger sized molecules, would result in an enhanced absorption of subcutaneously injected water-soluble molecules. Logically, one would expect an extravasation of molecules and fluid from the circulating blood into the interstitial tissues, resulting in tissue edema, as found during the local action of histamine and other permeability factors. However, histamine delays absorption (116, 117).

While the promoting action of hyaluronidase on the subcutaneous absorption of drugs, known and used for fourteen years, is highly significant the theoretical explanation of the mechanism is still in doubt. Likewise, the effects of other connective tissue factors on subcutaneous absorption are in doubt. Estrogenic

hormones in pharmacological doses reduce the permeability of the connective tissue, resulting in reduced spreading of fluid. Sprunt *et al.* (135) showed that the spread of India ink was reduced in rabbits treated with estrogenes. This has been reproduced by several authors, both *in vivo* (83) and in recently killed mice (27). Also, during pregnancy, which is followed by a physiological rise in the concentration of estrogenic hormones, a reduced spreading reaction has been demonstrated (36, 102, 134).

It would be natural to suppose that the reduced spreading of fluid in the interstitial tissue produced by estrogenic hormones would cause delayed subcutaneous absorption. Only a few experiments have explored this problem, and their results conflict. This may partly be due to the varied reactions of connective tissue to estrogenes in different species of experimental animals. An additional explanation may be the varied effect of the alterations in the connective tissue provoked by estrogenes on the absorption of different drugs and test substances. Hvidberg and Schou (71) found that the absorption of the non-electrolyte urethane was delayed in mice treated with estrogenic hormones. In these mice a pronounced rise in the amount of hexosamine in the skin and subcutaneous tissue has been demonstrated following estrogenic treatment (114). However, the absorption of sodium sulphacetamide from subcutaneous tissue in rats was found to be unaltered following treatment with estrogenic hormones; in this species, the concentration of hexosamine was unaltered after the hormonal treatment. As far as we know, these are the only investigations on the influence of estrogenes on absorption from subcutaneous tissue. In other localities of connective tissue conflicting results have been found. Ploman (102, 103) found an accelerated tissue clearance of intradermally injected uranin in pregnant women and rabbits. Jacox *et al.* (74) found a more rapid absorption of $^{24}NaCl$ injected intraarticularly in the knee in women as compared to men. Seifter *et al.* (129) showed that the absorption of phenolsulphonephthalein injected intraarticularly in rabbits was reduced following treatment with estrogenic hormones. As mentioned earlier, these results are not comparable because of the different species, the varying sites of connective tissue, and finally because of the different test substances and methods used in the investigations.

The absorption of urethane has been examined in mice in which the amount of connective tissue ground substance and the concentration of water had been altered by fasting and dehydrating the animals (69, 72). In both fasted and dehydrated mice, an accelerated absorption was demonstrated. When hyaluronidase was added to the injected solution of urethane, the difference between the experimental groups and controls was eliminated. In both fasted and dehydrated mice the amount of hexosamine in the subcutaneous connective tissue was reduced to the same degree (about 7%). While the water content in the fasted series was reduced about 12%, dehydration caused a much more pronounced reduction (33%). From these results the authors concluded that the concentration of water in the connective tissue is of little importance for the rate of subcutaneous absorption of a non-electrolyte such as urethane. The en-

hanced absorption in fasted and dehydrated mice was more probably produced by the above alterations in the amount of connective tissue ground substance. This corresponds well with the delayed absorption of urethane in estrogen-treated mice, in which the amount of hexosamine was increased while the water content was practically unaltered.

To judge from the foregoing investigations, the actual water balance and swelling capacity of the connective tissue seem to have no direct influence on the rate of subcutaneous absorption. However, an increased permeability of the connective tissue caused by hyaluronidase enhances the absorption rate of all dissolved substances. Especially for urethane, it has been shown that an increase in connective tissue ground substance is followed by a delayed absorption, while absorption is enhanced when the amount of hexosamine is reduced. We do not know if this is a general rule for the absorption of all dissolved substances, or if there are variations due to the physicochemical properties of the injected solutions. The water content of the connective tissue seems without influence on the rate of the subcutaneous absorption.

D. Self-depression of subcutaneous absorption

Self-depression of the subcutaneous absorption of drugs is the delay caused by endogenously liberated compounds, such as histamine and 5-hydroxytryptamine. Suggestive of this is the demonstration that the local addition of the antihistaminic agent, mepyramine maleate, and of 2-bromo-*d*-lysergic acid diethylamide (BOL 148), the antagonist to 5-hydroxytryptamine, to subcutaneously injected solutions of morphine greatly enhance its absorption (93, 95, 116, 117). It must be pointed out that self-depression has been demonstrated only in rats. We do not know if the phenomenon is found in other species. Because of its theoretical interest, a more thorough discussion of self-depression will be given.

Self-depression was first demonstrated for the absorption of sodium sulphacetamide, given in a nearly isotonic solution of physiologic pH. In addition to the accelerating effect of locally added mepyramine, an enhanced absorption was also found in rats depleted of their releasable histamine by treatment with compound 48/80. The local addition of histamine, on the other hand, depressed absorption. The theory was advanced that subcutaneous absorption is self-depressing, owing to histamine liberated by the injection-trauma and by the injection of a non-physiological fluid which acts as a foreign body (116, 117).

Since absorption was unaltered when BOL 148 was added to the solution of sulphacetamide, the author concluded that 5-hydroxytryptamine did not influence the absorption of sulphacetamide sodium. An even more pronounced enhancement of the rate of absorption was demonstrated when morphine was injected. The concentration of morphine in the blood was more than doubled when mepyramine was added locally or given systemically in histamine-blocking doses (95). This very pronounced self-depression of the morphine absorption can be explained by the potent histamine liberation caused by the drug itself.

With respect to the subcutaneous absorption of morphine, Milthers (93) has succeeded in showing that self-depression is not caused by histamine alone, but

also greatly by 5-hydroxytryptamine. Both depletion of tissue 5-hydroxytryptamine by systemically administered reserpine, and addition of BOL 148 locally in the solution of subcutaneously injected morphine enhanced the absorption to a very high degree. When 5-hydroxytryptamine was added, absorption was again delayed, returning to normal values.

In the same paper the author employed self-depression to explain the commonly known difference in morphine toxicity in very young and fully grown animals. The skin of young individuals contains much less histamine and releasable 5-hydroxytryptamine than does the skin of adults (54). In 12-day old rats the absorption of morphine injected subcutaneously on a weight-basis was much faster than in fully grown rats. Ten minutes after injection the concentration of morphine in the blood was almost six times higher than in fully grown rats. The local administration of mepyramine, BOL 148, histamine, or 5-hydroxytryptamine did not alter the absorption rate in the 12-day old rats. The explanation given for the high toxicity of morphine given subcutaneously to young rats was an enhanced absorption due to the total lack of self-depression. Not only is there lack of histamine and 5-hydroxytryptamine in the young individuals, but also the vessels of young rats do not react to histamine and 5-hydroxytryptamine as do those of fully grown rats. In a later study, Milthers (94) even showed that morphine infused intravenously is more toxic to fully grown rats than to very young rats. This further confirms the importance of the difference in absorption for the toxicity of morphine given subcutaneously to young and fully grown rats.

The above description of the absorption of morphine in rats at different ages is an example of the practical value of self-depression for the explanation of well-known biological phenomena. Unpublished investigations have shown that the more pronounced absorption from concentrated solutions than from dilute solutions containing the same dose of test substances (62, 122, 144) may be explained partially by a quantitatively higher self-depression when the dose is given in a larger volume. The differences in the rates of absorption from solutions of different concentrations are reduced when experiments are done on histamine-depleted animals.

Delay of the absorption caused by liberated histamine and 5-hydroxytryptamine may also explain the depression of subcutaneous absorption when injections are given using high injection pressures (6), or when injections are given following local heating of the absorption zone (7, 47, 49, 85, 86). Also, the enhanced absorption when repeated injections are given in the same zone may be another example. The first injection may produce a local depletion of the releasable histamine due to non-specific histamine liberation (16, 45, 76); the self-depression will therefore be reduced when later injections are given.

Further variations in self-depression, due to differences in the concentration of histamine and 5-hydroxytryptamine among areas of subcutaneous tissue, may explain the differences in absorption rate found in these areas (27). The condition of the connective tissue ground substance may, however, also influence the rate of absorption.

To date a final explanation of the delay of subcutaneous absorption produced

by histamine and 5-hydroxytryptamine cannot be given. The true capillaries are the only important part of the vascular system concerned with absorption from subcutaneous tissue. Our knowledge about the flow through true capillaries during inflammation, and about the mode of action of histamine and 5-hydroxytryptamine is insufficient. Although there is a dilatation of capillaries during inflammation this cannot be taken as a sign of enhanced flow. As early as 1877 Cohnheim (21) observed capillary stasis in experimental inflammation. Many authors have observed dilatation of capillaries simultaneously with a reduction of the total flow of blood through the vascular system during the action of histamine. Hilton and Holton (61) showed this in an isolated rabbit ear, just as Tripod and Wirz (143) demonstrated it in an isolated rabbit hindlimb preparation.

The total flow of blood from an artery to a vein in a certain area of the peripheral circulation cannot be taken as a definite measure of the capillary blood flow. We must know also the distribution of the total flow between the true capillaries and the arteriovenous shunts, and eventually other arteriovenous connections which do not affect absorption. Experiments by Hürlimann and Bucher (66) showed that histamine may alter the distribution of the blood flow between the true capillaries and arteriovenous shunts. In their experiments they used rabbit ears, adding lycopodium to the perfusion fluid (65). The spores having a diameter of about 30 μ occluded the true capillaries, but passed through the arteriovenous shunts, leaving them open for circulation ("anastomose ears"). When the action of histamine in different concentrations was examined at constant perfusion pressure in intact ears and "anastomose ears," it was found that low concentrations enhanced the flow, while higher concentrations reduced the flow. An interval of concentration existed where the capillary flow was reduced while the flow through the arteriovenous shunts was enhanced because of dilatation (65, 66). This concentration is comparable to that which is reached in the tissues following endogenous liberation of histamine. When the flow through the true capillaries is reduced, a delayed absorption follows irrespective of the total surface of the capillary membrane. The action of histamine on the permeability of the capillary wall and the effect on the paracapillary circulation may add to the histamine-induced depression of subcutaneous absorption. Histamine provokes the formation of tissue edema. During the production of edema, the resultant of the paracapillary circulation is directed from the capillaries outwards to the interstitial tissue producing a solvent drag, which further reduces absorption, even if the absorption is due only to diffusion.

The action of 5-hydroxytryptamine on the terminal circulation is also imperfectly understood. Further consideration is not warranted because the effect of liberated 5-hydroxytryptamine may be compared to the action of liberated histamine.

Subcutaneous injection of any fluid not physiologic with respect to ion-composition and pH, the trauma of the needle, and injection pressure all provoke a non-specific inflammation, as stressed by Bárány in 1932 (5). We know that histamine and 5-hydroxytryptamine are active when the early inflammatory exudate is produced (132, 133). This liberated histamine and 5-hydroxytrypta-

mine, the amounts of which vary with the histamine- and 5-hydroxytryptamine-liberating properties of the injected drug, produce a depression of subcutaneous absorption by an action on the terminal vascular bed. This self-depression may be reduced, thereby enhancing absorption, when histamine- or 5-hydroxytryptamine-blocking agents are added to the solutions injected, or when the releasable amounts of these compounds in the peripheral tissues are reduced after systemic treatment with histamine or 5-hydroxytryptamine liberators.

V. DRUGS INFLUENCING THE RATE OF SUBCUTANEOUS ABSORPTION

Various drugs and hormones given systemically or locally in the injected fluid in pharmacological doses can alter the absorption rate of subcutaneously injected drugs. The point of attack of such substances may be the connective tissue ground substance, the peripheral circulation in the zone of absorption, or the self-depression mechanism. Examples can be given of the different modes of action.

A. Epinephrine

Epinephrine was the first drug known to influence the rate of absorption of other drugs given subcutaneously. In 1903, Braun added epinephrine to subcutaneously injected solutions of carmine and cocaine (13, 14). The rate of absorption was determined both by the clearance of the coloured carmine from the local depot, and from the toxicity of the absorbed cocaine. Using other methods the effect of epinephrine on subcutaneous absorption was investigated by Okuneff (97) and Falck and Lange (44). The mechanism of the absorption-delaying effect of epinephrine is obvious, because the drug constricts the terminal vascular bed in the zone of absorption, constricting arteries, arterioles, capillaries, and possibly venules. Therefore, the flow of blood through the absorbing area, and especially the capillary flow, is depressed significantly, giving a reduced absorption. There is little destruction of epinephrine in the local area, which causes a prolonged effect of the drug locally (82). The practical value of epinephrine in connection with the administration of local anaesthetics need only be mentioned.

B. Hyaluronidase

The promoting effect of locally given hyaluronidase on subcutaneous absorption has already been mentioned in section IV, C. Hyaluronidase depolymerizes the hyaluronic acid of the connective tissue ground substance, which is followed by a reduced hydrophilic capacity, giving a more fluid ground substance and an increased permeability. Therefore, fluids injected together with hyaluronidase spread through a larger area of connective tissue and, accordingly, a greater number of capillaries and a larger total area of capillary membrane is exposed to the injected solution, giving an enhanced absorption.

It is still debatable whether hyaluronidase increases the permeability of capillaries. Duran-Reynals (38) showed that the extravasation of Evans Blue from the blood was accelerated by addition of a crude extract from testes. Aylward

(3) made the same observation, but it could not be reproduced by Rocha e Silva and Dragstedt (109) or Zweifach and Chambers (147). Too much weight cannot be attached to these experiments because crude preparations of hyaluronidase were employed. If Evans Blue is injected intravenously together with hyaluronidase, an increased rate of disappearance of the dye, followed by an increase in the concentration of serum-proteins and in hematocrit values, is noted (57, 58). Later Benditt *et al.* (10) denied that hyaluronidase increases the permeability of capillaries, because they found a decrease in the permeability-inducing action with increase in the purity of the preparations of hyaluronidase employed for the experiments. Possibly the concentration of hyaluronidase may be of significance for the results of such experiments because of the hyaluronidase-inhibiting capacity of serum, stressed by Hübner *et al.* (64). The edema produced when hyaluronidase is injected subcutaneously also points to a capillary permeability-increasing action (41, 75).

However, preparations containing hyaluronidase are still not chemically well defined. When preparations are able to produce increased capillary permeability, impurities containing capillary permeability factors, for example histamine, may be the cause. Braun and Weber (15) found edema production due to hyaluronidase in an isolated perfused hindlimb preparation. Simultaneously, however, the flow increased, even though the infusion pressure was held constant. This indicated that vasodilatation occurred in the preparation. This cannot be produced by hyaluronidase, but must have been caused by vasodilators, such as histamine. This applies also to the experiments by Szabo and Magyar (140), who demonstrated an increased flow in the thoracic duct simultaneously with a fall in hematocrit values. They explained this by an increased "shunting" of fluid from the blood through the extra-vascular tissues to the lymph, produced by a capillary permeability-increasing action of hyaluronidase. This may be produced by impurities, such as capillary permeability factors, because exactly the same experimental result was found earlier by the same authors when histamine was used (139). Spector (131) in his review also questioned the effect of hyaluronidase on the permeability of capillaries.

In conclusion, if hyaluronidase has a permeability-increasing action, as does histamine, it should cause a decrease in the rate of absorption. Therefore, it would antagonize the absorption-promoting effect of hyaluronidase produced by its permeability-increasing action on the ground substance of the connective tissue.

C. Estrogenic hormones

This subject has already been discussed in section IV, C. Systemic treatment with estrogenic hormones reduces the permeability of the connective tissue in certain species and localities, probably by increasing the hyaluronic acid content of the ground substance. This is followed by a slight, variable absorption-depressing effect. When estrogenic hormones decrease permeability of the connective tissue ground substance, then the absorption of non-electrolytes is depressed. It is not known whether the absorption of electrolytes is also depressed.

D. Adrenal glucocorticoids

Systemic treatment with cortisone and prednisone is followed by an increase in the rate of subcutaneous absorption. Again we must make the reservation that experimental demonstration has been limited to certain test substances and species of experimental animals. The first observation was made by using urethane as a toxicity test on mice (23). The time from the subcutaneous injection until loss of the righting reflex was taken as a measure of the rate of absorption. However, delay in this time following treatment with corticosteroids could be caused by a delayed elimination of urethane, or by an increased sensitivity to the narcotic action, provoked by cortisone. In experiments on rabbits, Schou (115), using cross-over absorption experiments, showed that subcutaneous absorption as evaluated by the blood concentration of the test substance was markedly increased following systemic treatment with cortisone. The results were reproduced in doubly nephrectomized rabbits (118) in order to exclude a possible effect of cortisone on elimination, that could simulate an enhanced absorption. Further, it was shown that cortisone treatment did not affect the concentration of sulphacetamide when this substance was infused intravenously at a constant rate (121); hence, the sulphacetamide space is unaltered during cortisone treatment. The absorption-promoting effect was reproduced with a water-soluble hydrocortisone compound given intravenously (120). Desoxycortisone has no influence on subcutaneous absorption (119).

A final explanation of the enhancing effect of cortisone and adrenal glucocorticoids on subcutaneous absorption cannot be given at present. The mechanism may be closely related to the anti-inflammatory and anti-edematous effect of adrenal glucocorticoids. During the anti-edematous effect there is not only a reduced production of inflammatory edema fluid, but also an acceleration of its reabsorption (23). Therefore, a solvent drag from the interstitial fluid to the circulating blood may exist during the action of cortisone when an artificial edema is produced by the injection of a drug solution into the subcutaneous tissue. This solvent drag may produce an enhanced absorption of dissolved molecules.

Furthermore, self-depression of subcutaneous absorption may be reduced after prolonged treatment with cortisone, which will also accelerate subcutaneous absorption. The synthesis of histamine is reduced in cortisone-treated animals (53, 112). Therefore, the rise in the content of histamine in the tissues following histamine depletion is delayed (52). It is only the synthesis and binding of histamine in the peripheral tissues that is reduced, while the spontaneous turnover is unaltered (111). Prolonged cortisone-treatment reduces the histamine content as well as the concentration of 5-hydroxytryptamine in the skin and subcutaneous tissue of experimental animals (59, 113). Desoxycortisone is without effect. Because of the reduction in the amount of releasable histamine and 5-hydroxytryptamine after prolonged treatment with cortisone, self-depression is reduced, giving an enhanced absorption. This explains also the more pronounced absorption enhancing effect of cortisone in cross-over experiments, because cortisone inhibits the replacement of the histamine liberated and elim-

inated during the first absorption control experiment (115). Possibly the sensitivity of the peripheral vessels to histamine is also reduced following cortisone treatment. This may further increase the absorption-enhancing effect of cortisone.

E. Antihistaminic agents

In section IV, D it was mentioned that blocking the vascular response to liberated histamine and 5-hydroxytryptamine by giving antihistaminic agents or 5-hydroxytryptamine-blocking agents locally or systemically may enhance subcutaneous absorption by decreasing the self-depression of absorption. A similar enhancement of absorption due to a reduced self-depression is seen in animals depleted of their releasable histamine and 5-hydroxytryptamine by treatment with liberators.

F. Diuretics

When edema is reduced by the action of a diuretic, the resultant net flow of the paracapillary circulation must be inwardly directed, giving an accelerated reabsorption of solutes in the edema fluid. It is questionable whether diuretics enhance the absorption from normally hydrated connective tissue. Engel and Epstein (42) described an accelerated disappearance of wheals during the action of diuretics. However, as mentioned in section III, wheal disappearance does not define absorption. In an investigation of the absorption of urethane from subcutaneous tissue in normally hydrated mice exposed to mersalyl treatment, Hvidberg *et al.* (70) found no effect on the absorption rate. Therefore diuretics probably do not affect subcutaneous absorption of drugs from normally hydrated animals.

VI. CONCLUSION

Our knowledge about connective tissue and characteristics of the ground substance has been greatly extended in recent years. However, our understanding of the subcutaneous absorption of drugs has not been significantly increased. The barrier to absorption is the capillary membrane. In addition to the qualities of this membrane, factors on both sides of the membrane influence the rate of absorption. The capillary blood flow is of major significance. This is not equivalent to the total blood flow through a part of the terminal vascular bed, because blood may be shunted from arteries to veins without passing through the true capillaries, the only vessels in which absorption occurs. There are no methods to determine to what extent the total blood flow passes through true capillaries. Until this problem is solved, we shall not be able to explain the mechanism of subcutaneous absorption.

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