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A PHARMACEUTICAL STUDY OF DRUG ABSORPTION

A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

By

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INTRODUCTION

Although the literature contains a great many reports on the administration of drugs, both subcutaneously and cutaneously, little work is reported on the type and rate of absorption.

In recent years, emphasis has been placed on the subcutaneous implantation of various medicaments, especially of the sex hormones. No reports have been observed as to the absorption of these medicinals, incorporated in various substances employed as ointment bases.

It appears that there is a need for study of the effects of ointment substances on the rate and type of absorption of medicinals.

Insulin was selected as the medicinal, because preliminary experiments showed that it was adapted to this type of study. The amount of insulin released to the blood stream could be determined indirectly by measuring the decrease in blood sugar levels at the necessary intervals.

Thus, this investigation was started with the realization of the need for additional study of the effects of substances employed as ointment vehicles on the type and rate of absorption.

A SURVEY OF METHODS EMPLOYED IN THE STUDY OF ABSORPTION

A. Subcutaneous

In recent years the study of subcutaneous absorption has been concerned with attempts to prolong the action of drugs by retarding their absorption. The problem of prolonging absorption has been studied by numerous investigators and applied to many different substances.

An early method used was the suspension of the medicament in an oily vehicle, which did not tend to spread through the tissues, and which delayed the absorption of particulate matter. This method was in practice for many years; for example, the intramuscular injection of mercurial and bismuthial compounds suspended in oil.

In 1929 Clauss B. Strauch[1] reported the prolonging effect of several drugs when injected in emulsion form. After several experiments he finally selected a method based on the following consideration: a water-in-oil emulsion had the property of enclosing the water with a film of fat. The subsequent absorption of the water was hindered by the thin films of oil that surrounded each drop of water.

Strauch proposed the theory that synthetically prepared emulsions were quickly destroyed when injected into the tissues unless some protective substance was added to the emulsion which would resist the action of the tissues.

The following is the formula he used: concentrated and sterile solution of the drug, 10 cc.; sterile olive oil, 3.5 cc.; sterile metacholestearin, 0.2 gm.; sterile myricin, 0.3 gm.

These were mixed and made into a sterile and stable water-in-oil emulsion. The author mentioned that the most effective protective substance he found was myricin (myricyl palmitin ester); and that the best emulgator he found was metacholestearin.

He began his experiments with a strong (1:50) solution of epinephrine using about 25% of its volume in olive oil. From 1 to 5 cc. of the total volume of the finished emulsion was injected intramuscularly. The result was a very gradual release of the epinephrine. Absorption of the drug occurred each time one oil film was released from the enclosed droplet of epinephrine. Strauch stated that the rate of this release was dependent on the amount of protective substance employed.

Other drugs employed to demonstrate this prolonged effect were: methylene blue, strychnine nitrate, lactose, and pituitary extracts. His most outstanding experiments were with insulin "repositories". Two dogs were made diabetic by complete removal of the pancreas, and were kept alive for 8 weeks with repository injections of 200 units of insulin given every 5 days. He also reported that more than 100 human patients were treated with insulin repository injections of from 100 to 1000 units of insulin each. Nine of these patients were treated over a period of 6 months. Injections were given every 5 to 7 days.

In 1934 Walsh and Frazer[2] reported on the effects of subcutaneous and intravenous injection of toxins combined with emulsions. They injected 2 rabbits subcutaneously with 5 minimum lethal doses of diphtheria toxin mixed with 0.5 cc. saline. At the same time a second group of 6 rabbits was injected subcutaneously with 5 minimum

lethal doses of diphtheria toxin mixed with 0.5 cc. of cod-liver oil emulsion. The 2 rabbits of the first group died within 36 hours while the second group showed no toxic symptoms and all survived. Walsh and Frazer concluded that the toxin-emulsion mixture was non-toxic when injected subcutaneously.

Brock and Druckery[3] in 1938 were able to demonstrate an effect lasting more than a year, by placing a collodion sac containing crystalline oestrone in the abdominal cavity of rats.

Another method of prolonging absorption was that of combining the compound with a protein, or with a protein and a heavy metal. Work in this direction was stimulated by the introduction of protamine and protamine zinc insulin, both of which proved of great value in the treatment of diabetes mellitus.

Protamine insulin was developed by Hagedorn and coworkers[4] in Copenhagen in 1936. It was a solution of insulin mixed with a solution of mono-protamine obtained from the sperm of a trout, Salmo irideus. The protamine insulin formed was precipitated. Subcutaneous fluids reacted to dissolve slowly the protamine insulin thus prolonging the action. Such a preparation gave greater efficiency per unit and made possible a lower dosage, the entire dose being given in the morning and lasting for the day. Protamine zinc insulin was still more prolonged in action. The requirement of protamine insulin was 15% less than that of standard insulin; that of protamine zinc insulin was 30% to 40% less. This compound was introduced by Scott and Fisher[5] in 1937 on the basis that zinc existed in the pancreas and was a constituent of crystalline insulin.

A more recent method of slowing absorption and prolonging the

action of drugs was the subcutaneous implantation of crystals and tablets of ovarian and other hormones. That a very prolonged effect of certain hormones could be obtained by the subcutaneous implantation of a compressed tablet of undiluted, crystalline substance was reported by Deanesly and Parkes[6], in 1937. They reported particularly favorable results with oestrone, oestradiol, progesterone, testosterone and desoxycorticosterone. This method allowed a very prolonged action for periods up to a year from a single administration and a very high effectiveness in relation to the total dose. The fact that months or a year might be required for the complete absorption of a tablet was due to the low solubility of the steroid substances in body fluids.

In 1938 Deanesly and Parkes[7] stated that there was no evidence to support the fact that the rate of absorption of the tablets was affected by the functional requirements of the animal.

This subcutaneous implantation method was also applied to tablets of commercial crystallin insulin by Parkes and Young[8] in 1939. They concluded from their experiments that insulin differed from many other physiologically active substances in that the hypoglycemic action of subcutaneously implanted tablets of insulin was only very slightly more prolonged than that of subcutaneously injected solution. They found that a connective tissue capsule surrounded some of the insulin implants.

Salmon, Walter, and Geist[9] in 1939 reported on the prolonged effect of implanted crystals of ovarian hormone in women. They pointed out that the high dosage of oestrogens in oil solution required to relieve the symptoms caused by ovarian failures was

attributed to the rapid absorption and excretion of the hormone. A total of 10 menopause cases was treated by implanting a-estradiol benzoate. A majority showed improvement as early as 6 days after the implantation. Complete relief of symptoms usually occured within 2 weeks and persisted to periods varying from 60 to 98 days. If the same amount of a-estradiol benzoate was administered in solution in oil, in a single dose, intramuscularly, it would produce only an incomplete effect both on the symptoms and vaginal smears. Unless repeated injections were administered the symptoms would recur in original intensity and tissues would regress to the pre-treatment stage 7 to 14 days after injection.

Because of these encouraging results obtained from the subcutaneous implantation of crystals and tablets of the sex hormones, this technique was applied to other crystalline hormones in the hope that this method of administration might result in an increase in their physiological effectiveness by prolonging their duration of action.

Bottomley, Folley, Walker, and Watson[10] discussed in 1939 the effect of subcutaneous implantation of adrenalin tablets on blood-sugar and milk composition in lactating ruminants. These adrenalin tablets, weighed up to 200 mg., and produced periods of hyperglycemia which lasted in most cases at least 42 hours.

Further work in applying this technique to other hormones was reported by Firor[11] in June of 1940 on the treatment of Addison's disease by the implantation of a synthetic hormone, desoxy-corticosterone acetate. He made over 30 implantations in treating 17 patients and found that the implants met the patients' requirements for

cortico hormone for periods ranging from 4 to 9 months.

Mark and Biskind[12] in 1940 confirmed the work of Parkes and Young as regards to crystalline zinc insulin, but further found that the addition of 20% protamine to the zinc insulin pellet greatly prolonged the effect obtained by implantation. A more comprehensive experiment was carried out by Cutting, Milton, and Cohn[13] in 1941 who made pellets of crystalline zinc insulin to which had been added various excipients such as cholesterol, cholesterol and lecithin, cholesterol and "entemb" (enteric coating), and charcoal. They found that equal parts of insulin and cholesterol with a minute amount of lecithin were the 2 most promising mixtures.

That only slight absorption took place from tablets of thyroxine implanted in dogs and men was reported by Greene and January[14] in 1940. However, Wokes[15] in 1941 obtained clear evidence of physiological effect by producing symptoms of hyperthyroidism in normal guinea pigs by the implantation of tablets of thyroxine. He found that the tablets had a very limited period of action, but this result was in sharp contrast to that obtained by Rowlands[16] in 1942. In 100 day experiments he found that a subcutaneously implanted tablet of thyroxine maintained the growth in thyroidectomized rats and he was unable to confirm Wokes results.

In an attempt to prolong the absorption of anterior pituitary extract, Busse[17] found in 1940 that tablets containing mixtures of sucrose, lactose, and starch were found to dissolve within several hours after implantation, and would not be of use in prolonging absorption over a long period.

Parkes[18] in 1942 presented a paper which described his investigation

into the possibility of adapting the implantation technique to the administration of non-steroid substances, with a special reference to the use of excipients. He found that a compressed tablet of a highly water-soluble substance made with or without an excipient was rapidly dissolved and absorbed by the subcutaneous tissues. An exception was cholesterol which he found to be very satisfactory.

He rejected the following excipients either because they disintegrated too rapidly or because they possessed no advantage over cholesterol: stearic acid, palmitic acid, uric acid, starch, kaolin, lumisterol, ergosterol, and sitosterol.

In the case of less water-soluble substances which in tablet form were absorbed slowly, Parkes thought that the excipient should be designed to facilitate rather than to delay absorption. No base was found.

Several authors pointed out the necessity of using great caution in the implantation of tablets in humans, since most of the tablets contained many times the therapeutic amount of the active ingredient. A crumbling of the tablet might prove dangerous.

Busse[17] incorporated an anterior pituitary powder in an aqueous ointment base and implanted a small portion of the mixture.

A base of glyceryl tristearate was prepared with which the pituitary powder was mixed. The water content of this base was 10 percent. The preparation gave excellent results. His results indicated that there was an optimum amount of water at which the greatest amount of stimulation would take place. Busse found that preparations having a concentration of 2.5 to 10 percent water gave the greatest degree of physiological response for this pituitary powder.

Fuller, Hawking, and Partridge,[19] in 1942, made an "in vitro" and "in vivo" study of the effect of various media upon the rate of absorption of sulfanilamide. From the "in vitro" experiments, it was found that absorption was much delayed by the incorporation of sulfanilamide in an oily base, or in a water-in-oil emulsion. It was not much influenced by incorporation in a glycerin-gelatin base or in an oil-in-water emulsion, or by particle size of the drug.

The "in vivo" experiments showed that if the preparation was spread as a thin film over a surface wound, absorption occurred rapidly from all types of preparations studied. When the preparation was embedded in the tissues as a compact mass, so that the surface to volume ratio was low, absorption of sulfanilamide was much delayed by incorporation in an oily medium and somewhat delayed by incorporation in an oil-in-water emulsion.

They found that soft paraffin delayed the action more than any other substance studied. Sulfanilamide in a cod-liver oil and beeswax base maintained an approximately constant rate of absorption, at a low level, for almost 7 days, as was indicated by urine analysis.

A method of studying subcutaneous absorption was described by Reiner, Keston, and Green[20] in 1942. They studied the absorption and distribution of insulin labelled with radioactive iodine. They found that the time of maximum rate of absorption, less than 2 hours, was soon followed by the maximum drop in blood sugar. It was also pointed out that as the absorption rate dropped to very low levels, the blood sugar rose to its original level.

BIBLIOGRAPHY

SUBCUTANEOUS

1. Strauch, C.B., J.A.M.A., 92, 1177, (1929).

2. Walsh, V.C. and Frazer, A.C., Brit. Med. J., 1, 424, (1934).

3. Brock, N. and Druckery, H., Klin. Wschr., 17, 23. (1938).

4. Hagedorn, H.C., Jensen, V. Krarup, N.B. and Wodstrup, I.A., J.A.M.A., 106, 177, (1936).

5. Scott, D.A. and Fisher, A.M., J. Pharmacol. 58, 78 (1937).

6. Deanesly, R. and Parkes, A.S., Proc. Roy. Soc. B., 124, 279 (1937).

7. Deanesly, R. and Parkes, A.S., Lancet, 2, 606, (1939).

8. Parkes, A.S. and Young, F.G., J. Endocrin., 1, 108, (1939).

9. Salmon, J.J., Walter, R.I. and Geist, S.H., Science, 90, 162, (1939).

10. Bottomly, A.C., Folley, S.J., Walker, F.H.A. and Watson, H.M.S., J. Endocrin., 1, 287, (1939).

11. Firor, W.M., Ann. Surg., 111, 942, (1940).

12. Mark, J. and Biskind, G.R., Endocrin., 26, 444, (1940).

13. Cutting, W.C., Milton, C.M., and Cohn, R.B., Endocrin., 28, 679, (1941).

14. Greene, J.A. and January, L.E., Am. J. Physiol., 129, 368, (1940).

15. Wokes, F., Quart. J. Pharm. and Pharmacol., 14, 165, (1941).

16. Rowlands, I.W., J. Endocrin., 3, 203, (1942).

17. Busse, L.W., Doctoral Thesis, U. of Wis. (1940).

18. Parkes, A.S., J. Endocrin., 3, 220, (1942).

19. Fuller, A.T., Hosking, F. and Partridge, M.W., Quart. J. Pharm. and Pharmacol., 15, 127, (1942).

20. Reiner, L., Keston, A.S. and Green, M., Science, 96, 362, (1942).

B. Cutaneous

Schafer[1] as early as 1898 realised the difficulties involving this problem when he wrote, "To decide the case for or against the possibility of absorption by the human skin, would appear a simple problem, yet a literature reaching back over a century indicates that the production of unimpeachable testimony on either side has proved a matter of no little difficulty".

According to Sutton[2] one of the first methods used to prove that absorption had taken place was by examination of the various excretions of the body, especially the urine, immediately following the application of the ingredient under trial.

Mussey[3], in 1809, mentioned that after topical application of certain drugs to the unbroken skin, these drugs were found in the urine. The list of the drugs was not given.

In 1876, Draschе[4] demonstrated that an alcoholic solution of salicylic acid applied to the skin caused the appearance of salicyl in the urine almost immediately. After the application of 20-30 grains of salicylic acid in olive oil to the axilla, Randolph and Dixon[5], in 1885, found that the urines of 6 patients gave positive results in iron tests for salicyl.

Bourget[6], in 1893, made a quantitative study of the effect of different fatty bases and the effect of such factors as age, sex and diseased conditions, on the cutaneous absorption of salicylic acid as judged by the excretion in the urine. He found that the rapidity and amount of absorption depended upon the vehicle. Lard and lanolin were the most effective, and vaseline and glycerol were

least effective. The skins of old individuals were found to be less permeable than those of the young. Absorption from skins of red haired and blond individuals was more rapid than that from dark haired subjects. The skins of females were more readily permeable than those of males.

The above method of urine analysis was employed by Neisser and Siebert[7] in 1904. They found the presence of mercury in the urine usually after the 5th day of application of their "Kalomel Salve".

Levin[8], in 1912, was not able to confirm the results of Draesche, Randolph and Bourget. His studies indicated that no salicyl appeared in the urine after the application of salicylate ointments to the skin.

In 1917, Wile and Elliot[9] worked with mercurial ointments. They reported that mercury salicylate inunctions were not easily absorbed. Ointments of red oxide of mercury appeared to be absorbed more rapidly than the yellow oxide ointments. Oleate of mercury was absorbed to about the same extent as mercury salicylate.

Schamber and coworkers[10] used the examination of excretion method, in 1918, when they concluded that rabbits might absorb some mercury by breathing, but that respiratory absorption was far less important than cutaneous absorption.

In 1924, Kahlenberg[11] reported that boric acid was found in the urine of individuals 55 seconds after they had immersed their feet in a solution of boric acid.

Leslie-Roberts[12], in 1928, concluded from his experiments that absorption took place from vaseline, alcohol, and water, but from the data one could not conclude definitely which of these solvents

was the most favorable for absorption.

Cole, Schreiber, and Sollmann[13] reported, in 1930, that the absorption of mercury by inunction depended directly upon the concentration of the metal in the base. The base, whether oleate, benzoated lard, suet, or stiff petroxolin, made little difference. They found that colloidal mercury ointments showed no greater excretion of mercury than the official ointments of equal concentration.

In 1931, Klenka[14] working with salicylic acid ointment, postulated that the base in which the medicament was applied influenced the rate and degree of absorption. He carried out experiments using as bases, vaseline, lanolin, liniment of ammonia and lanolin combined with camphor and with extract of capsicum, and showed that other constituents of the preparation had a marked effect on the absorption of salicylic acid. He reported that there was a more rapid and prolonged absorption when the ointments contained either camphor or capsicum, owing to the increased local circulation which resulted from the irritant effect of these substances. The presence of ammonia was found to lower the rate of absorption, owing to the formation of salicylamide.

Merz[15], in 1931, stated that it was generally known that free salicylic acid was readily absorbed through the skin, but that the statements published were somewhat contradictory. He showed that salicylates were absorbed both from a lanolin base and from a soap base.

Myiri and Jannitti[16], in 1932, after reviewing the literature which referred to the evenly matched controversy for the previous 65 years as to the skin absorption of iodine, stated that they

established the fact that iodine and iodides penetrated the unbroken skin. They reported that only 12 percent of the amount painted onto the skin was at the disposal of the skin; that 1 to 4 percent was the maximum taken up by the skin in the first few hours after application; and finally that at the end of 3 days, up to 5 percent of the total iodine was still within the skin. The remainder was absorbed later.

In 1934 and 1935 Bliss[17] [18] concluded that a drug which would be absorbed through the skin would be absorbed regardless of the vehicle. He believed that fatty or greasy ointment vehicles might exert the same retarding influence on drug absorption through the skin, that was demonstrated with these agents in the gastro-intestinal tract following oral administration.

Brown and Scott,[19] in 1934, made a study of the cutaneous absorption of methyl salicylate. They found that maximum absorption resulted from immersion of the hands in an aqueous suspension of 11.8 percent volume percent of the ester. Medial absorption resulted from immersion in a 80 percent solution in ethyl alcohol. The minimum absorption resulted from immersion in the pure ester.

Burgi[20] demonstrated, in 1937, that substances soluble in oil were more readily absorbed through the unbroken skin than those soluble in water.

In 1938 Zondek[21] reported that drugs in solvents such as alcohol, ether, benzene, and chloroform were more rapidly and more completely absorbed than those incorporated in an oily base.

After the discovery of insulin, several investigators attempted to introduce it into the body by inunction. The reports of the possibility of insulin absorption through the skin were conflicting.

Blood sugar analysis was the method used to determine the absorption of this drug.

Woodyatt[22], in 1922, said, "Experiments were conducted with oral, rectal, vaginal, intranasal, intravenous, and subcutaneous administration. Inunctions were also tried. Many variations were attempted in connection with each. Positive effects were obtained with subcutaneous and intravenous injection, very weak, doubtful or frankly negative results with the others."

In 1923 Rennie[23] found that the inunction of 100 units of insulin on a human diabetic had no effect.

Telfer[24], in the same year, reported that insulin could be introduced into the blood stream by inunction of large doses. He produced severe hypoglycemia in fasting rabbits by rubbing insulin ointments, of which lanolin and lard were the bases, into the shaved skin of the abdomen. Because he used a crude insulin, it was impossible to calculate the number of units employed. In one experiment convulsive seizures resulted; in another, hypoglycemia was still pronounced 12 hours after administration.

Harrison[25] concluded from his experiments, in 1926, that inunction of ointments containing insulin, even in large doses, was useless in clinical work as a substitute for subcutaneous injection. He used a lanolin base in an attempt to confirm Telfer's findings, but was unsuccessful with that, as well as with other bases.

In 1935 Hermann[26] and Hermann and Kassowitz[27] reported a marked fall of blood sugar in animals and patients following the application of an insulin salve to the skin. Details regarding the preparation and composition of this salve were not given.

Major[28], in 1935, and 1936[29,30], made numerous attempts to lower the blood sugar by means of "dermal" application to fasting rabbits. He used diethylene glycol monoethyl ether as the base for the insulin. He concluded that under proper conditions insulin might be absorbed through the skin of normal rabbits and might produce a definite and marked fall in blood sugar. His procedure was to rub the shaved abdomen of the rabbit with 3-4 drops of glycerin. Ten to 15 minutes later the insulin preparation was applied. After application, the solution was gently rubbed with a glass rod. He took care to avoid abrasions of the skin.

In 1936 Bauger and Flexner[31] came to the conclusion that, "The absorption of insulin by the skin of rabbits is dependent upon the integrity of the integument. The intact skin shows little or no absorption, whereas a recently abraded skin, such as produced by shaving, permits the absorption of an appreciable amount of insulin."

Starkenstein and Hendrych[32], in the same year, demonstrated that insulin could be absorbed by the skin from an undisclosed ointment providing the cholesterol was first removed from the skin by some solvent such as petroleum ether and other organic solvents. They also showed that saponin added to the preparation increased the absorption of the insulin.

In 1937 Major and Delp[33] stated that their experiments showed that cutaneous absorption of insulin occurred in rabbits and in patients, independent of abrasions produced on the skin. They concluded, however, that the absorption was very inconstant.

Haffter[34], in 1942, applied an ointment containing 30-40 units of insulin to the skin of rabbits. He concluded that insulin, neither

Reiss[36], in 1936, performed experiments using cholesterol, lanolin, stearic acid, a vegetable oil and soap. He concluded that the skin might be a "two-way passage"*. He injected the material "intracutaneously" and after 3-4 days, the portion of the skin injected was excised and examined histologically. The biopsy specimens were frozen and sectioned in a microtome, and then stained. All of the fats tended to migrate toward the epithelium. He found that cholesterol was "resorbed" most quickly and that stearic acid was "resorbed" most slowly.

Eller and Wolff[37], in 1939, employed this method of microscopic analysis in their investigations. They judged the degree of penetration by the amount of fat seen along the hair follicles and within the sebaceous glands. They demonstrated that liquid fats permeated the skin more rapidly than solid fats. It was shown that animal fats had the greatest depth of penetration. Vegetable fats and "mineral fats" followed in that order. Eller and Wolff concluded that most of the fats showed optimum penetration 4-6 hours after application. After 6 hours, the quantity of fat in the deeper tissues appeared to diminish.

Physiological reactions, as a means of determining whether absorption had taken place, were used by Macht[38] in 1932-33. He made a study of the relative penetration of fats and oils through the skin of animals, particularly rabbits and guinea pigs. Ointments were made of petrolatum, lard, hydrous wool fat, a synthetic proprietary preparation of cholesterol, goose fat, bear fat, and grease-less creams. Lotions were prepared with olive oil, cotton-seed oil, lard oil, mineral oil and peach kernel oil. The penetration of several potent drugs such as nicotine and strychnine, incorporated

* See Theories Concerning Mechanism of Absorption.

in these bases or vehicles, was studied pharmacologically.

His experiments revealed that none of the fixed fats carrying potent drugs were absorbed readily but that signs of poisoning developed in some cases, only after repeated application of the ointments for several days. Hydrous wool fat was more efficient than the other fats, but, on the whole, the results obtained with drugs incorporated in fats as well as in olive oil, linseed oil and liquid petrolatum were disappointing. He reported that the fixed oils and fats penetrated the normal epidermis to a very slight extent. Some drugs incorporated in the fats were absorbed less readily than the same drugs applied in aqueous or hydro-alcoholic solution.

Macht pointed out that most of the essential oils applied in sufficient quantity, were readily absorbed through the skin. Strychnine, dissolved in a little alcohol and mixed with any one of theessential oils, applied to the skin of mice and rats, was carried into the deeper tissues and produced typical strychnine poisoning. The fixed oils gave no such effect. Oil of orange and oil of wintergreen were two of the oils employed in this experiment.

Much work has been done on the application of sex hormones to the skin, proving their absorption by effects on the genital organs, such as cornification of the vagina, increased size of a breast, or increased weight of prostate glands and seminal vesicles.

Zondek[39], in 1929, showed that oestrone was absorbed by the skin. He found that 7 times the amount which produced vaginal cornification by injection, was necessary to do so by inunction of the hormone when incorporated in oil or ointment, to the shaved skin of mice.

MacBryde[40], in 1939, showed that in women, the inunction of an oestrogenic ointment to one breast was followed by a greater response in the treated breast, than in the other breast which received the ointment base only.

Dennesly and Parkes[41], in 1937, concluded that testosterone and testosterone acetate or propionate, when given by inunction in oil or propylene glycol, were not highly effective in restoring the prostate and seminal vesicles of castrated male rats.

In 1938 and 1939 Voss[42,43] compared the effects of testosterone and testosterone propionate, administered by injection in oil and superficial application in ointment and in 96% alcohol, on a eunuch, a eunuchoid, and a case of delayed puberty. He concluded that 2-3 times the injected dose of testosterone propionate must be applied as an ointment, and about 6 times the injected dose as a tincture in alcohol, to obtain comparable results.

Emmens[44], in 1940, stated that the volatile organic solvents, ether, benzene, and 96% alcohol, were more effective as carriers of active material from the skin surface than was nut oil or lanolin. In tests with rats and mice, inunction in benzene or ether was as effective as, or slightly more effective than, injection in oil. In tests with rabbits, inunction in benzene, but not in ether, was superior to injection in oil. Emmens also postulated a slight, but consistent, superiority of ether as a medium for inunction of oestrogens, and of benzene for inunction of androgens.

Cutaneous administration of vitamins was reported, in 1934, by Amrhein[45]. He demonstrated the cure of rickets in animals and human beings by the cutaneous application of cod liver oil or ointments

containing irradiated cholesterol. He stated that the cure was due to the absorption of vitamin D.

Kasahara[46], in 1937, showed that vitamin C was percutaneously absorbed. This was evidenced by the increased amounts of vitamin C in human milk after the application of an ascorbic acid solution to the intact skin of the mammae.

Common colds were cured when pro-vitamin A, factor 3, extracted from "carotene" was rubbed on the skin. This was developed by the Llwellyn Biological Institute[47] of West Los Angeles. The hundred and fifty patients with early colds were massaged with 15 drops of the extract on the skin of each inner thigh or with 30 drops on the abdomen. It was reported that 109 patients recovered from their colds and 23 others showed marked improvement. No results were observed in the remaining 18 cases.

BIBLIOGRAPHY

CUTANEOUS

1. Schafer, E.A., Text. Physiol., 1, 685, (1898).

2. Sutton, R.L., Brit. Med. J., 1, 1225, (1908).

3. Mussey, R.D., Phil. M. and Phys. J., 2, 288, (1809).

4. Drasche, Wien. Med. Wochnschr., 26, 1049, (1876).

5. Randolph, M.A., and Dixon, S.G., Med. News, 46, 174, (1885).

6. Bourget, Therap. Monatshf., 7, 531, (1893).

7. Neisser and Siebert, Med. Klin., 1, 9, (1904).

8. Levin, E., Deutsch. Med. Wochn., 38, 2412, (1912).

9. Wile, U.J. and Elliot, J.A., J.A.M.A., 68, 1024, (1917).

10. Schamberg, J.F., Kolmer, J.A., Raiziss, G.W., and Gavron, J.L., J.A.M.A., 70, 142, (1918).

11. Kahlenberg, L., J. Biol. Chem., 62, 149, (1924).

12. Leslie-Roberts, H., Brit. J. Derm. and Syph., 40, 325, (1928).

13. Cole, H.N., Schreiber, M., and Sollmann, T., Arch. Derm. and Syph., 21, 372, (1930).

14. Kionka, H., Klin. Wschr., 34, 1570, (1931).

15. Merz, K.W., Arch. Pharm., 269, 449, (1931).

16. Myrii, W. and Jannitti, M., J. Pharm. and Exp. Therap., 50-51, 32, (1932).

17. Bliss, A.R., Jr., Drug and Cos. Ind., 35, 3, 247, (1934).

18. Bliss, A.R., Jr., Ibid., 36, 730, (1935).

19. Brown, E.W. and Scott, W.C., J. Pharm. and Exp. Therap., 50-51, 373, (1934).

20. Burgi, E., Schweiz. Med. Wchnschr., 67, 433, (1937).

21. Zondek, B., Lancet, 1, 1107, (1938).

22. Woodyatt, R.T., J. Met. Res., 2, 793, (1922).

23. Rennie, J.K., Brit. Med. J., 2, 450, (1923).

24. Telfer, S.V., Brit. Med. J., 1, 715, (1923).

25. Harrison, G.A., Quart. J. Med., 20, 187, (1926).

26. Hermann, S., Arch. f. Exp. Path. u. Pharm., 179, 529, (1935).

27. Hermann, S. and Kassowitz, H., Ibid., 179, 524, (1935).

28. Major, R.H., J. Lab. and Clin. Med., 21, 278, (1935).

29. Major, R.H., Am. J. Med. Sci., 192, 257, (1936).

30. Major, R.H., Soc. Exp. Biol. and Med., 34, 775, (1936).

31. Bruger, M. and Flexner, J., Proc. Soc. Exp. Biol. and Med., 35, 429, (1936).

32. Starkenstein, E. and Hendrych, F., Arch. f. Exp. Path. u. Pharm., 182, 664, (1936).

33. Major, R.H. and Delp, M., Soc. Exp. Biol. and Med., 37, 338, (1937-38).

34. Haffter, Schweiz. Apotheker-Zeit., 8, 101, (1942).

35. Gardiner, F., Brit. Med. J., 1, 238, (1912).

36. Reiss, M., Acta. Dermat. Venerol., 17, 112, (1936).

37. Eller, J.J. and Wolff, S., Arch. Dermat. and Syph. 40, 900, (1939).

38. Macht, D.I., J.A.M.A., 110, 409, (1938).

39. Zondek, B., Klin. Wschr., 2, 2229, (1929).

40. MacBryde, C.M., J.A.M.A., 112, 1045, (1939).

41. Dennesly, R. and Parks, A.S., Proc. Roy. Soc. B., 124, 279, (1937).

42. Foss, G.L., Lancet, 2, 1284, (1938).

43. Foss, G.L., Ibid., 1, 502, (1939).

44. Emmens, C.W., J. of Endocrin., 2, 368, (1940-41).

45. Amrhein, F.J., J.A.Ph.A., 23, 182, (1934).

46. Kasahara, M. and Kawashima, K., Klin. Wchnschr., 16, 135, (1937).

47. ----------, Drug and Cos. Ind., p. 555, May, (1942).

SURVEY OF MATERIALS USED IN THE STUDY OF ABSORPTION

	Reference
URINE ANALYSIS	
Acid Acetylsalicylic	(1)
Acid Boric	(2)
Acid Citric	(2)
Acid Hydrochloric	(2)
Acid Salicylic	(3)
Acid Sulfuric	(2)
Bronyl Salicylate	(1)
Constituents of Volatile Oils: (such as thymol, eugenol, amyl alcohol, pinene)	(4)
Iodides	(5)
Iodine	(5)
Iodoform	(6)
Mercuric Chloride	(4)
Mercuric Salicylate	(7)
Mercurous Chloride	(8)
Methyl Salicylate	(9)
Oleate of Mercury	(7)
Red Oxide of Mercury	(7)
Salicyl Salicylate	(1)
Sulfanilamide	(10)
Turpentine	(6)
Yellow Oxide of Mercury	(7)
HUMAN MILK ANALYSIS	
Vitamin C	(11)
FECES ANALYSIS	
Cod Liver Oil	(6)
BLOOD ANALYSIS	
Adrenalin	(12)
Constituents of Volatile Oils: (such as thymol, eugenol, amyl alcohol, pinene)	(4)
Insulin	(13)
Salicylic Acid	(14)
Sulfanilamide	(10)
GENITAL RESPONSE	
Female	
Anterior Pituitary	(15)
Oestrone	(16)
Male	
Testosterone	(17)

	Reference
TREATMENT OF DISEASE	
Rickets	
Vitamin D	(18)
Colds	
Pro Vitamin A, Factor E	(19)
Pyrexia	
Creosote	(6)
Guaiacol	(6)
Ichthyol	(6)
PHYSIOLOGICAL-PHARMACOLOGICAL ACTIONS	
Diaphoretic	
Pilocarpine	(6)
Diuretic	
Digitalis	(6)
Emetic	
Apomorphine	(4)
Morphine	(4)
Local Anesthetic	
Cocaine	(20)
Opiates	(4)
Miotic	
Pilocarpine	(20)
Mydriatic	
Atropine	(20)
Cocaine	(20)
Toxic	
Arsenic	(4)
Belladonna	(6)
Essential Oils	(4)
Constituents of Volatile Oils: (such as thymol, eugenol, amyl alcohol, pinene)	(4)
Hydrogen Cyanide	(21)
Lead	(4)
Nicotine	(4)
Phenol	(4)
Strychnine	(4)

BIBLIOGRAPHY

1. Kienka, E., Klin. Wschr., 34, 1570, (1931).

2. Kahlenberg, L., J. Biol. Chem., 62, 149, (1924).

3. Draschi, Wien. Med. Wschi., 26, 1049, (1876).

4. Macht, D.I., J.A.M.A., 110, 409, (1938).

5. Myiri, W. and Jannitti, M., J. Pharmacol. and Exp. Ther., 45, 85, (1932).

6. Reilly, T.F., J.A.M.A., 36, 250, (1901).

7. Wile, U.J. and Elliot, J.A., J.A.M.A., 68, 1024, (1917).

8. Neisser and Siebert, Med. Klin., 1, 9, (1904).

9. Brown, E.W. and Scott, W.C., J. Pharmacol and Exp. Ther., 50-51, 32, (1934).

10. Fuller, A.T., Hawking, F. and Partridge, M.W., Quart. J. Pharm. and Pharmacol., 15, 127, (1942).

11. Kasahara, M. and Kawashima, K., Klin. Wschr., 16, 135, (1937).

12. Bottomly, A.C. et al, J. Endocrin., 1, 287, (1939).

13. Hermann, S. and Kassowitz, H., Arch. f. Exp. Path. u. Pharmak, 179, 529, (1935).

14. Kimura, Giiti, Oriental J. Diseases Infants, 27, 7, (1940).

15. Busse, Ph.D. Thesis, U. of Wis., (1940).

16. Zondek, B., Lancet, 1, 1107, (1938).

17. Foss, G.L., Lancet, 1, 502, (1939).

18. Amrhein, F.J., J.A.Ph.A., 23, 182, (1934).

19. ----------, Drug and Comm. Ind., p. 355, May, (1942).

20. Leslie-Roberts, H., Brit. J. Dermot. and Syph., 40, 325, (1928).

21. Walton, D.C. and Witherspoon, M.G., J. Pharmacol. and Exp. Ther., 26, 315, (1925-26).

PROPOSED THEORIES CONCERNING THE MECHANISM OF ABSORPTION

Reid[1], in 1892, carrying out experiments on living frog skins to determine the mechanism of absorption, stated, "It is possible to have a true absorption process taking place without the aid of ordinary osmotic action".

In 1904, Neisser and Siebert[2] indicated that absorption of mercury was through the hair follicles. After application of several mercurial ointments, they found particles of mercury in the hair follicles. Gardiner[3] in 1912, made a study of the penetration of several ointment bases. He concluded that the bases were absorbed through the hair follicles.

Wile and Elliot[4], in 1917, drew several conclusions from their experiments with mild mercurous chloride ointment and other mercurial ointments. The mode of absorption of mercury salicylate was both by volatilization and by direct absorption. Non-volatile salts were absorbed through the skin, but elimination and absorption were far slower than in the case of salts having a high vapor pressure. The more rapid appearance of mercury in the urine, in the case of the volatile salts, was probably due to the combined action of volatilization and inhalation through the lungs and absorption through the skin.

Kahlenberg[5], in 1924, conducted unique experiments working with boric acid, lithium chloride, rubidium chloride, cesium chloride, strontium chloride, lithium perborate, and sodium perborate.

"Both feet were washed with hot water and a little castile soap, thoroughly rinsed repeatedly with warm, distilled water and finally with 90 percent alcohol. After being wiped with a clean cloth, they were then immersed to a depth just above the ankle in the solution to be tested. The latter was kept at 45°C. in a 5 gallon stoneware jar which served as the foot bath. The feet were kept resting but lightly and not flatly, on the bottom of the jar, and they were also moved about occasionally. Samples of urine were taken from time to time, and analyzed."

It was found that free boric acid alone passed through the living skin in perceptible quantities. He stated, "The solution of the latter probably is adsorbed by the skin, loosely combined with the latter, and from this loose combination the boric acid is swept away by the blood stream".

All of the substances tested passed readily through dead human skin and through other dead animal membranes. These substances also passed through the living mucous membranes of the mouth and alimentary canal. Kahlenberg stated that since experiments on osmosis demonstrated conclusively that the chemical nature of the membrane was the factor determining whether a substance would pass through the membrane, he was led to the conclusion that dead skin and living skin were chemically different, for they acted quite differently osmotically. Therefore, the living mucous membranes were chemically different from the living skin since they acted differently osmotically. These two septa were similar when dead in that all of the substances studied passed through both, but when living, the skin was penetrated by boric acid only, the mucous membranes were penetrated by all of the dissolved substances.

Leslie-Roberts[6] reported in 1928 that pure synthetic salicylic acid could be transported through the epidermis into the connective

tissues and thence into the blood stream. He believed that the drug was retained by the colloids of the connective tissues by adsorption, and from these surfaces it was liberated gradually, passed into the blood, and was mainly excreted by the kidney. The permeability of the skin to salicylic acid varied in different individuals according to the "physico-chemical condition of the inner environment". Concerning this environment, he stated, "These conditions cannot be defined in the present state of our knowledge but there is some evidence which suggests that the key to penetration is held by the fibroblasts of the cutaneous connective tissue." He also pointed out that in respect to the mechanism of absorption, it might be said that although solid keratin was itself quite impervious to salicylic acid, it nevertheless played an important part in the translocation of the acid through the skin. It acted through its extraordinary power of adsorbing the acid whereby a supersaturated solution was formed on the surface of the skin. It was from this surface layer that the fraction transported was drawn and not from the mass of solvent. The chemical form in which the acid was transported was still open to discussion.

According to Leslie-Roberts[6] the strong affinity of salicylic acid for sodium rendered it probable that sooner or later the sodium salt was formed. The researches of Bekstein and Wile[7] pointed out the ease with which cholesterol formed esters with salicylic and other aromatic acids. They suggested the possibility that salicylic acid passed as a lipoid ester.

Kienka[8], in 1931, reported his study of the mechanism of absorption through the skin. He regarded the epidermis as a cellular membrane

saturated with fats containing cholesterol and believed that only substances which were soluble in these fats could diffuse through the epidermis. He mentioned that salicylic acid possessed this property and in addition it exerted a solvent action on the cutin, thus facilitating absorption. He also postulated that the base in which the medicament was applied influenced the rate and degree of absorption.

As to the mechanism of percutaneous absorption, Lasareff, Prussilovskaya and Livschitz[9], in 1931, found that solvents such as benzene, ether, and acetone were themselves absorbed into the blood stream when inunated on the ear of a rabbit, or on the human skin. They suggested that these solvents might therefore carry a dissolved hormone with them.

In 1932 Sollmann[10] discussed conditions influencing absorption. He stated that the time required for absorption into the body and into the cells ranged from a few seconds with HCl to several weeks with lead. Rapidity of absorption depended upon the nature of the drug, the place of administration, and a number of accessory factors. He mentioned that only soluble substances could be absorbed, but the solubility in the protein containing fluids of the body was not necessarily the same as the solubility in water. The solubility might also have been modified by chemical changes produced by the tissue juices.

Bliss, in 1934[11] and 1935[12], employed the histology of the skin to explain his conception of skin absorption.

"The intact human skin offers almost perfect protection against brief contact with aqueous solutions. The major defense of the human skin against penetration is found in its physical structure. The highly compact layers of stratified squamous epithelium, a stratum of keratinized cells, and the coating of fairly waterproof sebum afford rather excellent protection under ordinary conditions. Prolonged maceration by warm, moist application partially breaks down the defense and the macerated skin surface absorbed much more readily than the dry skin.

"The lining of the sebaceous glands of normal human skin practically constitute the only living cell layer whereby substances may be absorbed when applied to the skin. Because of the fact that the glands are filled with fat, penetration into or through the skin is limited apparently to fats, greases, and oils, fat-soluble and fat-solvent substances and hydrophil bodies incorporated with fatty vehicles.

"Inunction which forces the material into the ducts of the sebaceous glands, hyperemia of the area induced by friction, heat or irritation, occlusive dressings, and the preliminary removal of sebum by scrubbing with soap and warm water materially aid penetration. If the dead horny layer of the skin is removed or its continuity interrupted, absorption takes place through it more readily. Softening the layers of the epidermis increases the size of the interstices between cells, and may thus facilitate penetration. Fatty substances also inhibit the evaporation of sweat and thus favor maceration and softening of the skin."

He pointed out that the site of application, thinness of skin, freedom from hair, and richly supplied lymphatics, as on the flexor surfaces of the body, were aids for absorption.

Other conclusions that he made were: the drug itself rather than the vehicle in which it was applied was the determining factor of absorption; young subjects, females with soft fine skin texture, blondes, and obese people who perspired freely and enhanced softening and maceration had about 5 percent more prompt andintensive reactions; susceptibilities might have been due to differences in anatomical structure of epidermis or of sebaceous and sweat glands, to special

characteristics of the sebaceous matter and possibly to differences in the formation of protein, fatty and cholesterol compounds with the drugs themselves.

Brown and Scott[13], in 1934, pointed out that it was generally considered that a substance must possess both oil and aqueous solubility in order to penetrate the human skin and that, "an extremely high or extremely low partition coefficient is less conducive to absorption than intermediate values". They demonstrated that massage increased absorption as did an increase in temperature. They found that the type of base had no effect on the quantity of substance absorbed.

In the same year McCord[14] published an article calling attention to the inadequacy of the data available in industrial disease. He made the following statement which might be applied today, "The mechanisms by which many substances accomplish passage through the skin are little known. On an empiric basis, it has become established that numerous medicaments and intoxicants penetrate the skin; and, conversely, that others are barred. With the possible exception of fat-dissolving agents, the entering substances are often so dissimilar as to stultify any belief in the commonness of properties permitting absorption." He also stated that the entire situation was such that only by trial might the fate of any agent with an unknown status as to skin absorbability be determined.

The author set up the following factors as influencing skin absorption:

"1. Sustained, profuse sweating eventuating in an alkaline perspiration, may deprive the skin of its oily protection, and facilitate skin absorption.

2. Circumstances leading to an hyperemia of the skin promotes skin absorption.

3. Breaks in the integument, such as from a dermatitis or trauma, favor entry into the body. Such entry may not, however, constitute true skin absorption.

4. Fat-dissolving agents, such as naphtha may themselves enter the body or create opportunity for other substances to find entry through the skin.

5. Friction applied to the skin, such as the inunction of mercury ointments is conductive to skin absorption.

6. Failure to free the body of contact with materials that may enter the skin is related to the practical dangers of skin absorption of industrial intoxicants.

7. Naturally oily skin offers additional difficulties to the entry of some substances.

8. The younger skin, the greater the probability of skin sorption by that particular skin, up to the years of senility, and in the absence of skin injury.

9. Cataphoresis may thrust into the skin substances not otherwise absorbable."

McCord postulated that no one physical process governed the passage of chemicals through the skin; that osmosis was a probable explanation of how boric acid passed through the skin; that adsorption might play a significant role with HCN; and that diffusion, molecular size, surface tension, and capillarity most likely had an important role in determining skin absorption.

McCord's theory proposed that the characteristic shared by all substances that penetrated the normal skin was the capacity for "cell wetting". This activity of cell wetting was far more complex than was implied in the simple example of water being barred from

the skin by the oily film there present, and the function of soap in breaking down this interposed incompatible layer. The behavior of mercury ~~was~~ in contact with metallic lead furnished a more acceptible concept of "surface wetting". If metallic mercury was placed in contact with a piece of lead, the mercury entered and diffused in all directions. In time the lead became crumbly, or at least brittle. Electrical tests proved that the lead was unchanged so what happened was that the mercury, having the peculiar property of "wetting" the crystals of lead seeped in between all the faces of the crystals, and in time made the lead mass crumbly by depriving the lead of its intercrystal cohesion. The relationship between lead and mercury was not equally true for mercury and iron, or mercury and cadmium. Applying this "crystal wetting" to "cell wetting" it became possible to understand how some substances might pass between successive layers of epidermal cells until brought in contact with those deeper skin layers richly supplied with blood and lymph vessels. He pointed out that in the corium absorption was readily accomplished.

In 1936 Reiss[15] injected fat-like bodies intracutaneously. He mentioned that this method was hardly normal, but that it was discussed for what it might contribute to an understanding of the mechanism of the skin. The evidence produced indicated that the epidermis commenced at once to break up the fat. The author discovered that in every case the first result was a separation of the fat from its solvent, then a grainy dispersion and phagocytosis, and finally the migration of the fat toward the epithelium in cells designed as "lipophore cells", accompanied by a chemical transformation

of these fats. These processes were said to cause a change in keratinization. Injecting different fats, variances in resorption were observed. Reiss found that these artificially introduced fats traveled toward the surface of the skin. The same direction of travel was followed by nutrients obtained by eating and delivered by the blood stream after digestion to the subcutaneous tissues, which in turn fed the epidermis.[16] He pointed out in other experiments that fats could also travel in the opposite direction, that the epidermis might be a two-way passage for fats and it might be "entirely reasonable and logical to introduce fats from the outside if the processes which proceed from the inside out lie down on the job for any reason, such as ill health, old age, etc." Cutaneous absorption would seem then to be a process of reversing nature.

The role of cholesterol in the skin was studied by Starkenstein and Hendrych[17] in 1936. They attributed to cholesterol a permeability regulating action and concluded that it must exercise a protective action since an insulin ointment which failed to produce any blood sugar lowering when rubbed on the normal skin of a rabbit, produced a startling drop in the blood sugar level if the skin was previously washed with petroleum ether, a solvent for cholesterol.

Miller and Wolff[18] demonstrated in 1939 that fats permeated the skin and that they did so largely along the hair shafts and into the oil gland ducts.

BIBLIOGRAPHY

THEORIES OF MECHANISM OF ABSORPTION

1. Reid, E.W., Brit. Med. J., 1, 323, (1892).

2. Neisser and Siebert, Med. Klin., 1, 9, (1904).

3. Gardiner, F., Brit. Med. J., 1, 238, (1912).

4. Wile, U.J. and Elliot, J.A., J.A.M.A., 68, 1024, (1917).

5. Kahlenberg, L., J. Biol. Chem., 62, 149, (1924).

6. Leslie-Roberts, H., Brit. J. Derm. and Syph., 40, 325, (1928).

7. Eckstein, H.C., and Wile, U.J., J. Biol. Chem., 69, 181, (1926).

8. Kionka, H., Klin. Wschr., 24, 1570, (1931).

9. Lasareff, N.V., Krassilovskaya, A.I., and Livschitz, F.B., Arch. Gewebepath. Bewebehyg., 2, 641, (1931) through J. Endocrin., 2, 370, (1941).

10. Sollmann, T., A Manual of Physiol., (1932).

11. Bliss, A.R., Jr., Drug. and Cos. Ind., 35, 247, (1934).

12. Bliss, A.R., Jr., Ibid., 36, 730, (1935).

13. Brown, E.W. and Scott, W.C., J. Pharm. and Exp. Ther., 50-51, 373, (1934).

14. McCord, C.P., Am. J. Pub. Health, 24, 677, (1934).

15. Reiss, H. Acta. Dermat. Venerol., 17, 112, (1936).

16. ----------, Givaudian, Feb. (1942).

17. Starkenstein, E. and Hendrych, F., Arch. f. Exp. Path. u. Pharm., 182, 664, (1936).

18. Eller, J.J. and Wolff, S., Arch. Derm. and Syph., 40, 900, (1939).

INTRODUCTION TO EXPERIMENTAL PART

A previous study of absorption which was carried out in cooperation with Dr. R. K. Meyer established the importance of regulating the rate of absorption of drugs administered subcutaneously. An extract of sheep anterior pituitary powder, when incorporated with a base such as glyceryl tristearate and implanted in 21 day old female rats, resulted in an increase in the weight of the ovaries from a normal of 13 mg. up to as high as 126 mg.

From these results it was proposed that a study be made of the absorption of medicinals from various bases. The purpose of these experiments was to determine the effect of the base on the rate and type of absorption of the medicinal.

Insulin was found to be a more suitable agent to use in studying the effect of the base on the rate and type of absorption than anterior pituitary powder, because the concentration of insulin released to the blood stream from the base could be determined indirectly by measuring the decrease in blood sugar levels at necessary intervals.

Blood sugar determinations were carried out using Folin's micro-method.[1] The sugar was oxidized with alkaline potassium ferricyanide, and the ferrocyanide produced was measured colorimetrically as Prussian Blue.

Female rabbits, weighing between 1.8 and 2.2 Kg. were used as test animals. Between experiments they were kept on a diet of hay and oats but were not fed 24 hours previous to the experiments.

The function of protamine zinc insulin was to prolong the

action of insulin. It was successful and clinical experience proved its value. It was thought to the point to run experiments with this preparation to compare the absorption from this type of preparation to the absorption from various bases. Many attempts were made to complete an experiment with this preparation. All attempts in which larger units were employed resulted in convulsions; with the 0.5 unit it was possible to have the blood sugar level return to its original level. A dilution of 0.5 cc. of U-40 protamine zinc insulin in 9.5 cc. of sterile distilled water was made, and 0.25 cc. of this dilution, containing 0.5 unit, was injected. See Experiment 1.

Redgrove[2] reported that, "Glyceryl monostearate by itself is completely useless for the production of creams of the oil-in-water type. It is not an oil-in-water emulsifier. It is not colloidally soluble in water, and it does not disperse in the menstruum unless a dispersing agent is also present. Formulas presented are completely unworkable with the pure substance. The explanation is that workers who have recorded their results have used not commercially pure glyceryl monostearate but certain commercial mixtures, apparently in complete ignorance of their composition and the part played by glyceryl monostearate in the production of emulsions of the oil-in-water type."

He also stated that soaps must be present to form the emulsion.

The glyceryl monostearate used in experiment 2 was a commercial mixture containing glyceryl monostearate, sodium stearate and free stearic acid. It was impossible to obtain its exact composition.

The original plan was to incorporate the insulin crystals with

this base containing varying percentages of water (anhydrous, 5 p.c., 10 p.c. and 20 p.c.), and to study the absorption of these preparations. The consistency of the base, however, did not lend itself to the incorporation of the crystals with it. If standard insulin solutions were added to glyceryl monostearate, an ointment-like preparation resulted and the insulin was taken up by the base.

The implantation method required anesthesia and the discomfort of minor surgery. A more practical method would be that of injection. To do this, a syringe, modified to deliver these ointment-like preparations was constructed.

Pure glyceryl tristearate like pure glyceryl monostearate is not an emulsifying agent. The substance used in Experiment 3 was a commercial mixture containing soaps to allow the base to take up water and to form a water-in-oil emulsion. It was not possible to obtain the exact composition of this substance. Both glyceryl tristearate and glyceryl monostearate are called "self-emulsifiers" because of the property of taking up water in the presence of soaps and becoming emulsified; the former results in a water-in-oil type emulsion and the latter in an oil-in-water type emulsion. One might expect a longer duration of action from a water-in-oil emulsion since it would be less miscible with tissue fluids, than from an oil-in-water emulsion which would be more miscible with aqueous fluids.

The tristearate base did not emulsify the solution of insulin as well as did the monostearate base. Upon forcing the preparation out of the modified syringe the emulsion "broke" and insulin separated from the mixture. Therefore, it was impossible to duplicate

results obtained in any one experiment.

Since the injection method proved unfavorable with the tristearate base, it was decided to observe the effects of subcutaneous implantation using zinc-insulin crystals in place of the solution. These crystals were not employed in the injection method because the consistency of the substances, without the presence of water, made it impossible to use the modified syringe.

In analyzing the experimental results, the objection of uneven and irregular absorption from these mixtures became apparent. This might have been due to the physical qualities of the bases used, for upon examination of the implant at the completion of the experiment, it was observed that it was no longer a homogeneous mass, but granular in nature.

Further experiments using this series of bases were abandoned temporarily and efforts to find a base which could permit a more even absorption of insulin were carried out.

Petrolatum is probably the most frequently used ointment base for topical application. It is considered to be the least penetrating of any of the bases used. No attempts to use the vehicle subcutaneously were observed in the literature. Therefore, experiments were carried out to determine its value in prolonging the absorption of insulin.

Preliminary experiments showed that 1 mg. and 0.5 mg. zinc-insulin crystals incorporated with the petrolatum were too much and the majority of rabbits went into convulsions. It was then found that 0.3 mg., 7.2 units, incorporated with 100 mg. of petrolatum was satisfactory.

Experiment 4 shows these results and also the results from attempts to slow the initial drop from normal by coating the preparation with wool fat, ~~petrolatum~~ paraffin, glycyl monostearate and collodion.

Aquaphor is another frequently used ointment base for topical application. It is essentially petrolatum with 6 percent cholesterol esters isolated from wool fat incorporated with it. It forms emulsions of the water-in-oil type with water or aqueous solutions or medicaments. Experiment 5 is a series of experiments in which zinc insulin crystals were incorporated with aquaphor containing varying percentages of water.

In order to give petrolatum a firmer consistency, 25 percent paraffin was added. See Experiment 6. This base did not exert any beneficial effect in prolonging the absorption.

A base was then prepared consisting of petrolatum, 26.5 ~~percent~~ parts; aerosol*, 0.5 ~~percent~~ part; and cetyl alcohol, 8 ~~percent~~ part. This preparation melted approximately at body temperature and was capable of absorbing water. It resulted in an oil-in-water type emulsion. It was thought that a preparation of this type would be miscible with the body fluids and become emulsified, thereby making it possible to allow a continuous absorption from the outside to the center of the mixture. The absorption was even, but was no longer than the control.

Glycerite of tannic acid, because of its protein precipitating action, was added to this preparation in an attempt to prolong the action.

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* American Cyanamid and Chemical Corp., Bridgeville, Pa. "Ester of Sulfonated Dicarboxylic Acid".

See Experiment 6.

3

Fuller, Hawking, and Partridge reported on the absorption of sulfonamides from standard wounds and found that a mixture of cod liver oil and white beeswax prolonged the absorption. Such a base was prepared to observe its effect on the absorption of insulin from the subcutaneous tissue. See Experiment 6.

BIBLIOGRAPHY

INTRODUCTION TO EXPERIMENTAL PART

1. Folin, O. J. Biol. Chem., 77, 421, (1928).

2. Redgrove, S. H., The Perf. and Ess. Oil Rec., 33, 84, (1942).

3. Fuller, A.T., Hawking, F. and Partridge, M.W., Quart. J. Pharm. and Pharmacol., 15, 127, (1942).

EXPERIMENTAL PART

SUBCUTANEOUS

Experiment 1

Controls

Formula	Initial Drop	Length of action
1. Normal	---	---
2. Standard Insulin Sol. 5-U	4	8
3. Zinc-Insulin Crystals 24-U	4	12
4. Protamine-Zn Insulin 0.5-U	4	24

Normal blood sugar values were determined at intervals in order that the normal variation of the blood sugar level might be observed. Results of this determination on 4 rabbits are shown on curve I and formula 1. These curves indicate that the variation in the normal blood sugar level of a fasting rabbit was slight, being not more than 10 mg. percent. Any variation greater than 10 mg. percent was attributed to the action of the insulin.

Five units of standard insulin solution, formula 2, was injected subcutaneously and blood sugar determinations were made every 2 hours until no more effect was noticed on the blood sugar level. The results from this work on 3 rabbits are also shown on curve II. These were used as controls for experiments in which standard insulin solutions were employed. This curve shows that the duration of action on the blood sugar level of 5 units of standard insulin was approximately 8 hours.

As a control for experiments where crystalline insulin was used, 24 units of zinc-insulin crystals*, formula 3, were implanted in the

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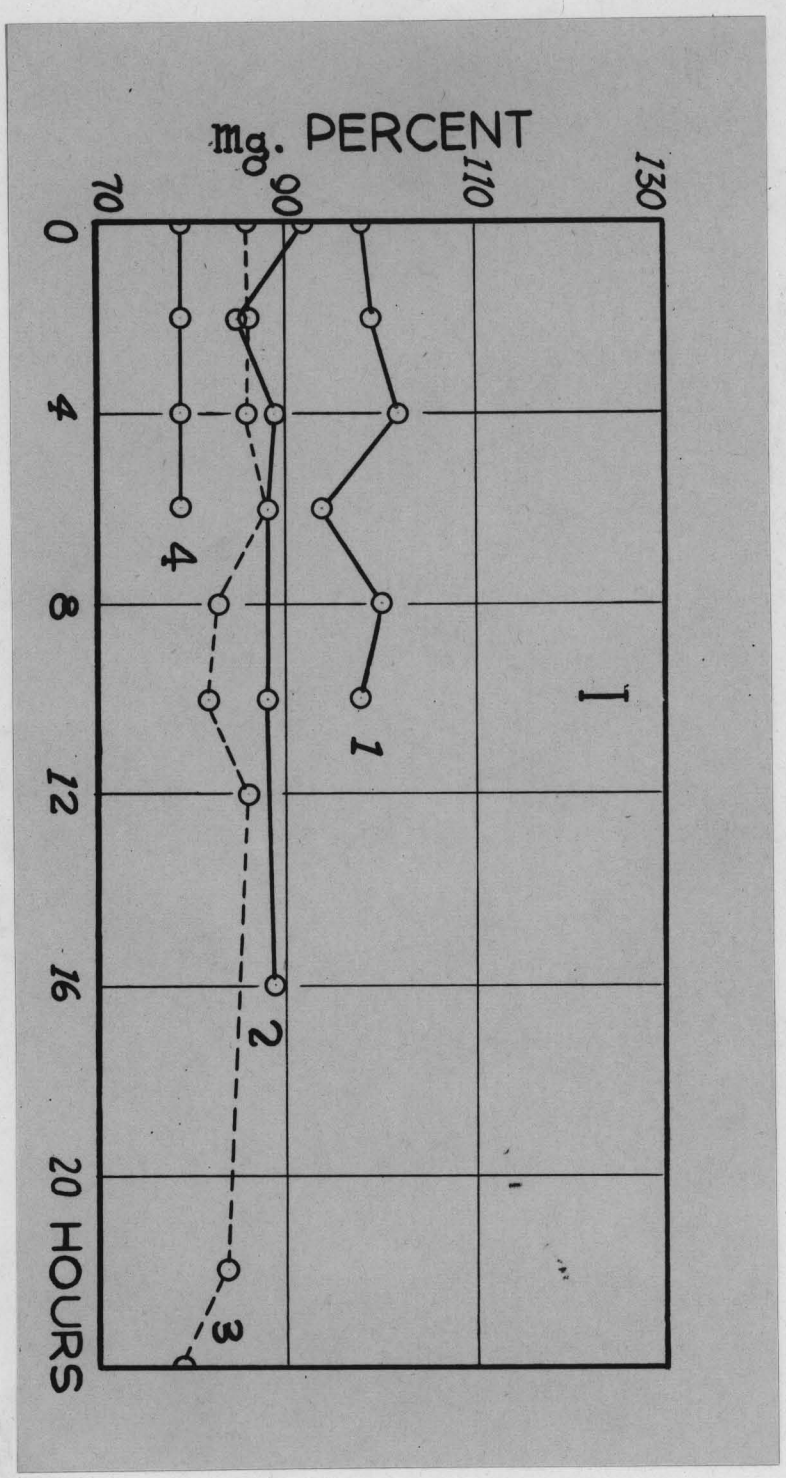
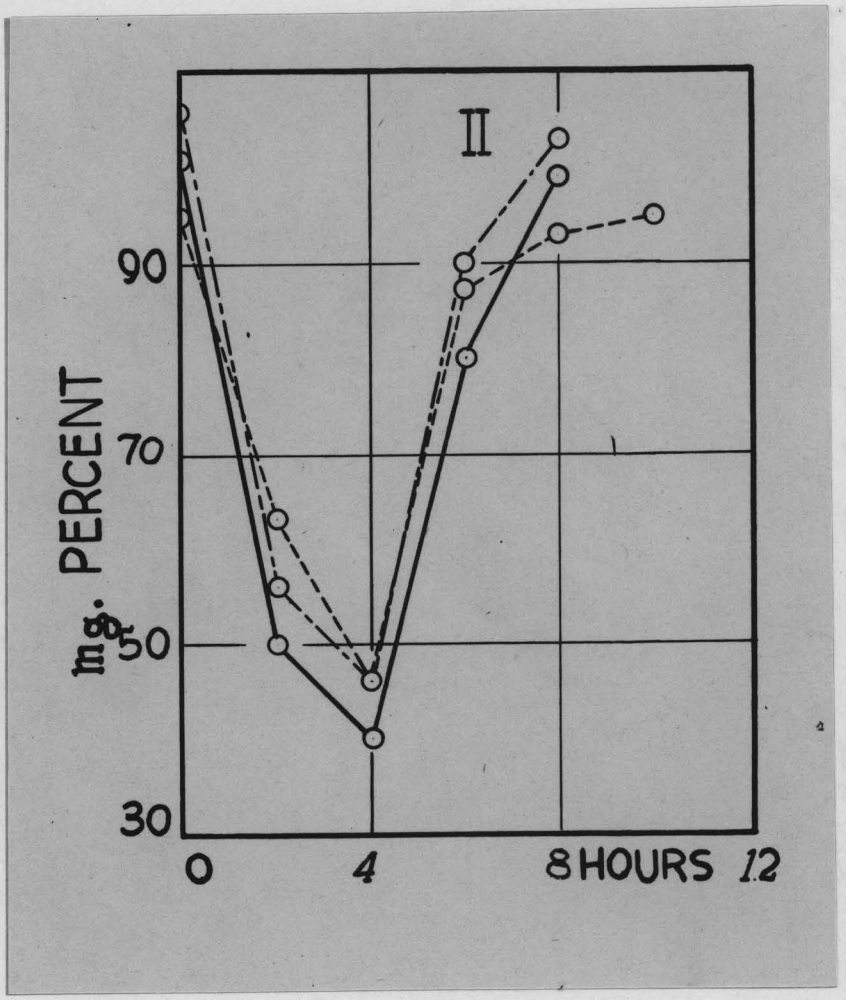
* Eli Lilly and Co., Indianapolis, Indiana. 1 mg. equals 24 units. 1 mg. was the smallest amount that could be implanted with accuracy.



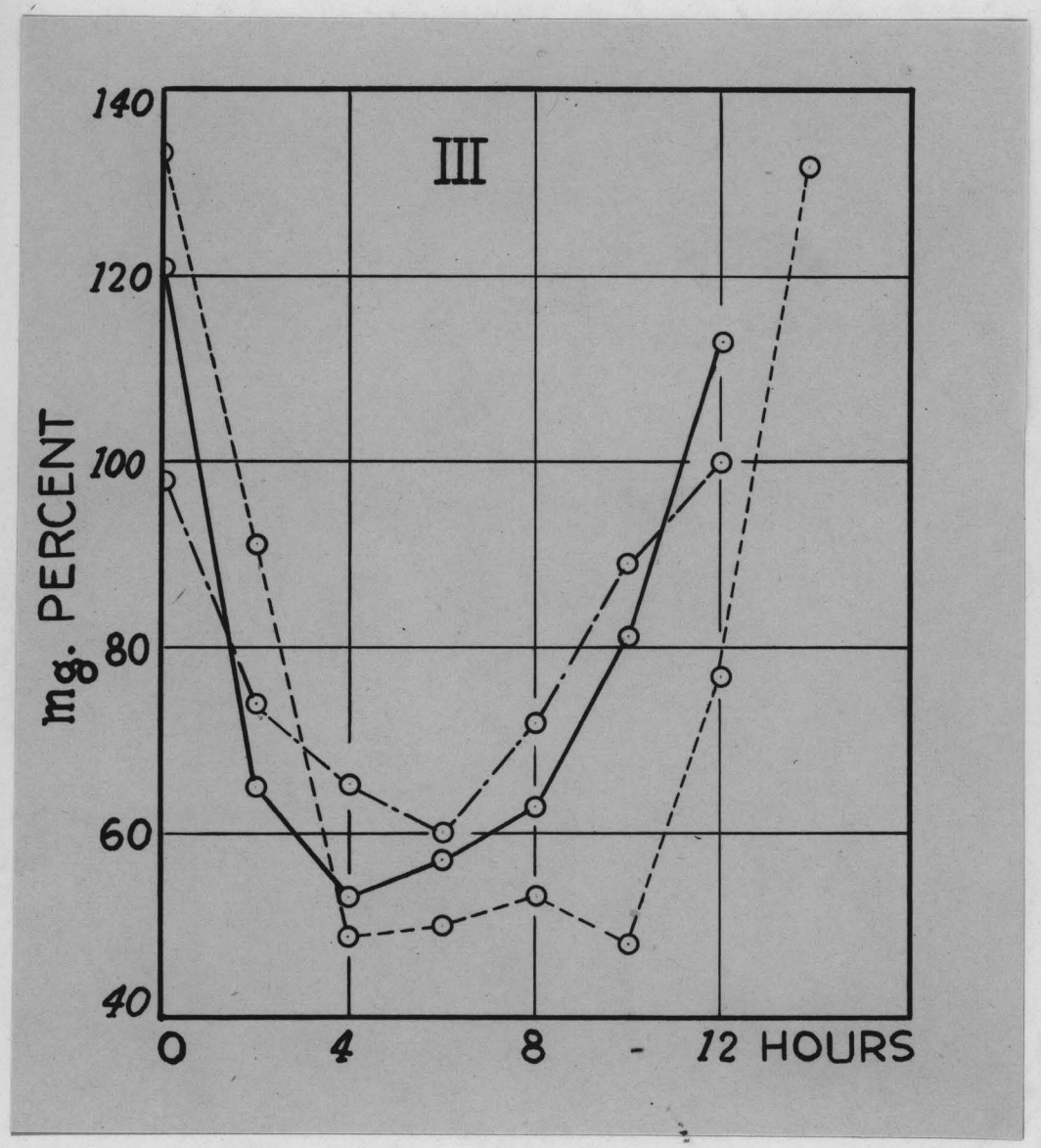
Normal

Note: Each curve on the following graphs represents one animal reaction.



Standard Insulin Solution

5 Units



Zinc Insulin Crystals

1 mg.

24 Units

flank of 3 rabbits. The area was first anesthetized with a 2 percent solution of procaine hydrochloride in normal saline solution to which was added 1 percent epinephrine (1:1000) just before its use. An incision about 1 inch in length was made with a scalpel and a pocket in the subcutaneous tissue was formed with a blunt forceps. The crystals were placed into this pocket and the opening sutured.

The results of the crystalline insulin control are also shown on curve III. The duration of action was 12 to 14 hours. If in using these crystals in a base, absorption longer than this period resulted, the prolongation was assumed to be due to the base.

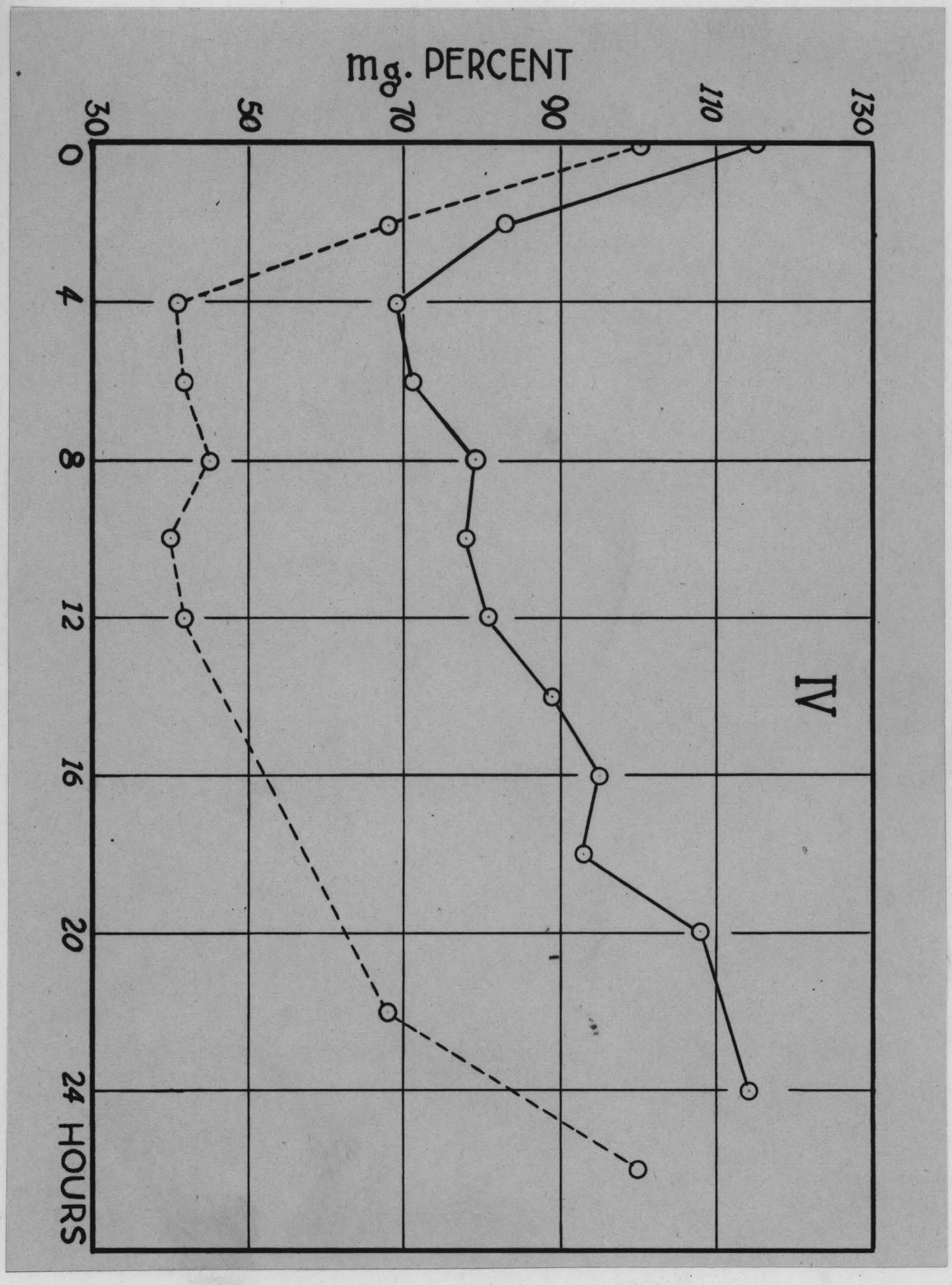
The absorption from 0.5 unit of protamine zinc insulin is shown on curve IV and also in formula 4. The duration of action was shown to be approximately 24 hours. The curve indicates that the initial drop in the blood sugar level was rapid, but it remained low for 6 to 8 hours and then gradually returned to its original level.

Experiment 2

Glyceryl Monostearate*

Formula	Injection: Initial Drop hours	Injection: Length of action hours
5. Glyceryl monostearate - 2.0 gm. Insulin Solution - U-20-2.0 cc.	2	16-18
6. Glyceryl monostearate- 1.9 gm. Water (5%)---------- 0.1 gm. Insulinsolution U-20--- 2.0 cc.	2	12-14
7. Glyceryl monostearate- 1.8 gm. Water (10%)---------- 0.2 gm. Insulin Solution U-20- 2.0 cc.	2-4	12
8. Glyceryl monostearate- 1.6 gm. Water (20%)---------- 0.4 gm. Insulin Solution U-20- 2.0 cc.	4	12-14

* Glyco Products, Inc., New York City.



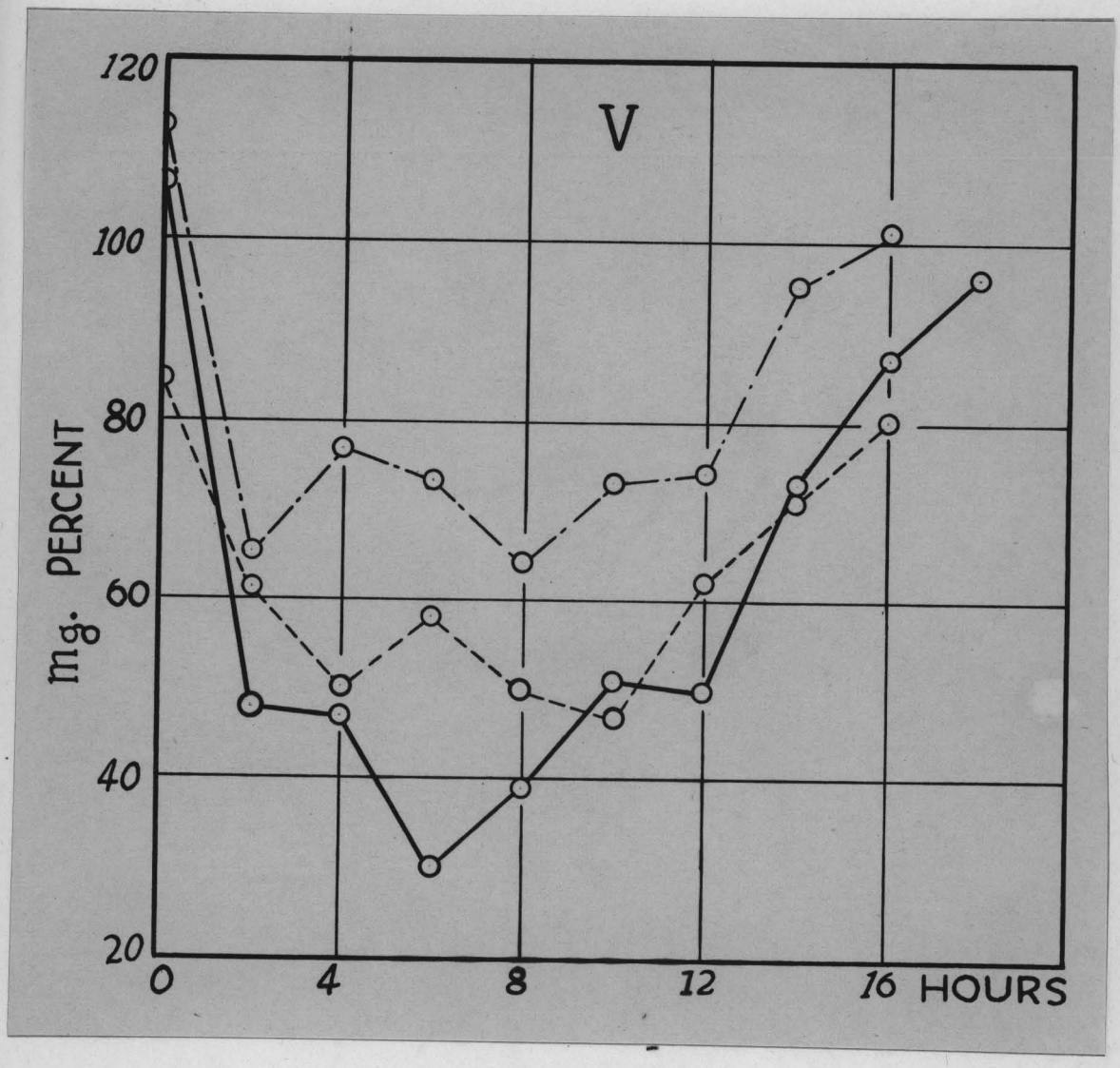
Protamine-Zinc Insulin

0.5 Unit

This series of experiments employed preparations in which 40 units of standard insulin solution was incorporated into a glyceryl monostearate base containing varying percentages of water, formulas 5,6,7 and 8. These preparations were injected subcutaneously so that each rabbit received 5 units of insulin. The results of this series are also shown on curves V, VI, VII and VIII.

These curves indicate that the effects of these preparations lasted from 12 to 18 hours, longer by 4 to 10 hours than the control. (See curve II). The prolonging of the absorption concerned the return to normal of the blood sugar level and not the initial fall which was similar to that of the control. The rapid descent of the blood sugar during the first few hours might have been due to the absorption of the readily available insulin on the surface of the preparation; the maintaining of a low level might have been due to the absorption of insulin from within the base.

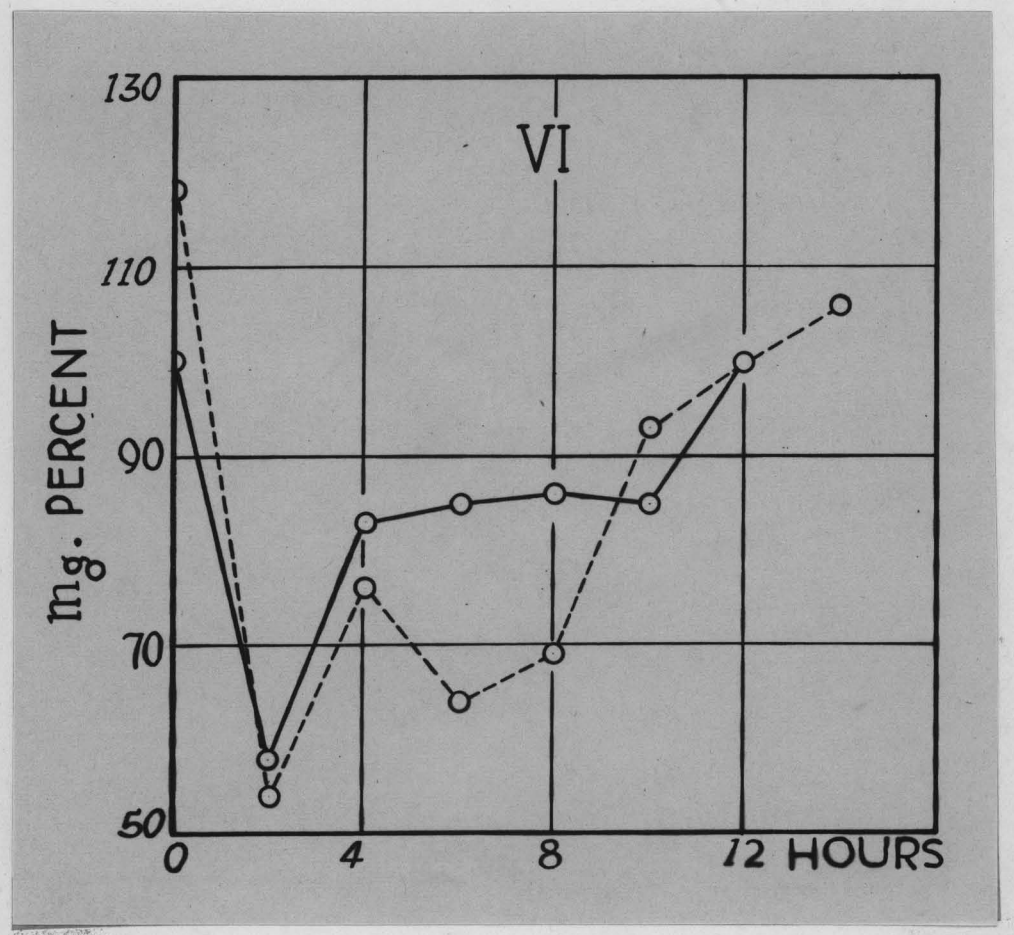
The duration of action was longest with formula 5, curve V. A possible explanation of why the duration of action was less with formulas 6, 7 and 8 was that these bases became more miscible with the tissue fluids and allowed the insulin to be absorbed too rapidly from the base. A preparation with lower water content might make it more difficult for the insulin to be liberated and result in a more even, prolonged absorption.



Glyceryl Monostearate
Anhydrous

Standard Insulin Solution

5 Units

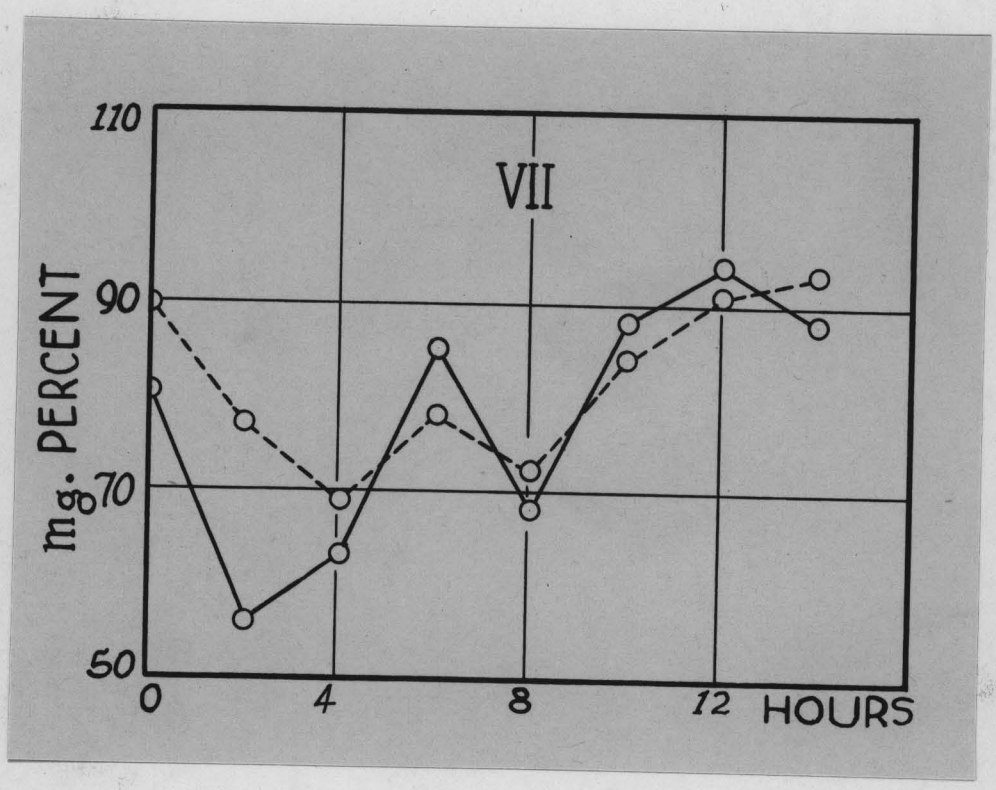


Glyceryl Monostearate

5 p.c. Water

Standard Insulin Solution

5 Units

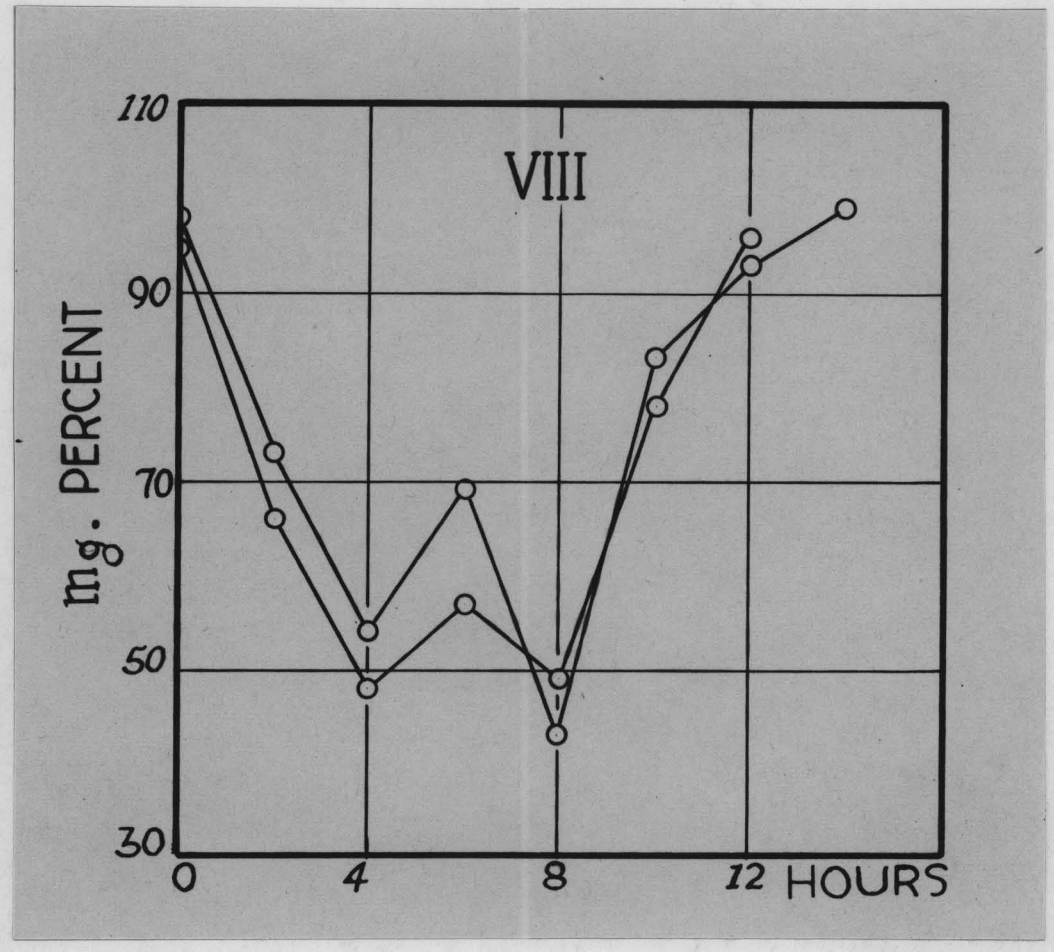


Glyceryl Monostearate

10 p.c. Water

Standard Insulin Solution

5 Units



Glyceryl Monostearate

20 p.c. Water

Standard Insulin Solution

5 Units

Experiment 3

Glyceryl Tristearate *

Formulas		Implantation: Initial Drop hours	Implantation: Length of action hours
9. Glyceryl tristearate -	0.100 gm.		
Zinc-insulin crystals	0.001 gm.	8	28-40
10. Glyceryl tristearate -	0.095 gm.		
Water (5%)	0.005 gm.	8	16-20
Zn-insulin crystals---	0.001 gm.		
11. Glyceryl tristearate -	0.090 gm.		
Water (10%)	0.010 gm.	6-8	32-40
Zn-insulin crystals---	0.001 gm.		
12. Glyceryl tristearate -	0.080 gm.		
Water (20%)	0.020 gm.	2	16
Zn-insulin crystals---	0.001 gm.		

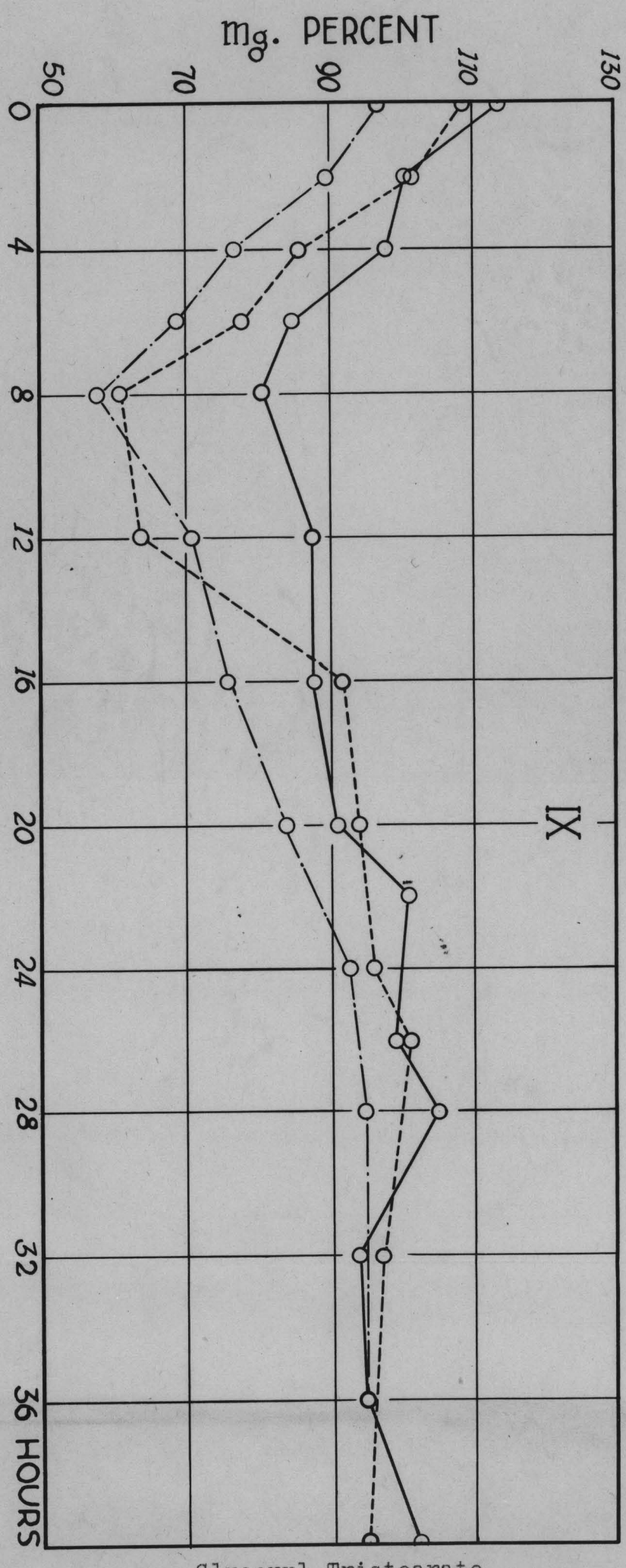
This series of experiments employed preparations in which 1 mg., 24 units, of zinc-insulin crystals was incorporated into a glyceryl tristearate base containing varying percentages of water, formulas 9, 10, 11 and 12. Curve III represents the control for these experiments. The technique was the same as described under the control.

The results of this series are also shown on curves IX, X, XI and XII.

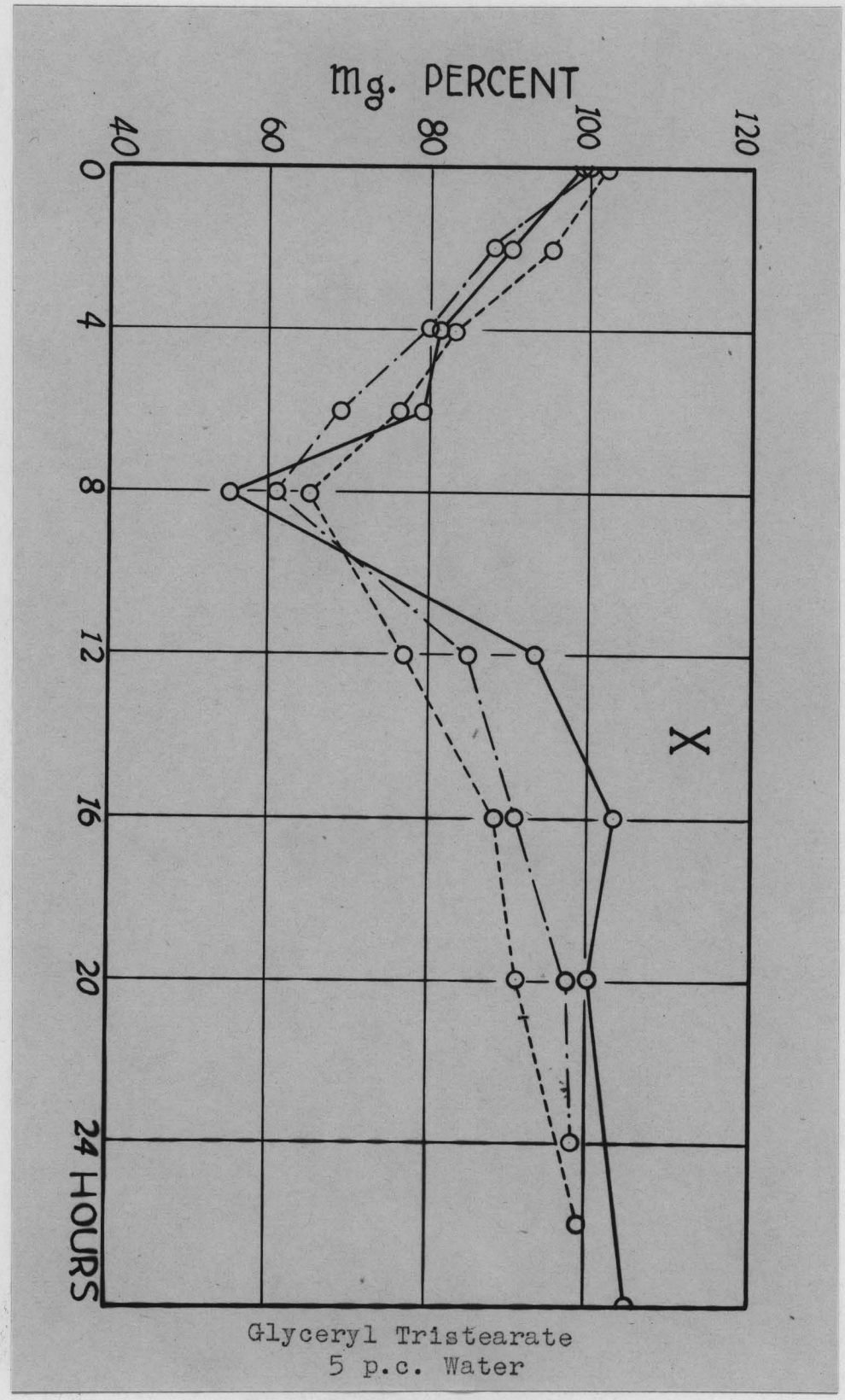
With formula 9 the duration of action of the insulin was 28 to 40 hours. There was a rather even fall from normal. This fall, as well as the return to the original level, was more prolonged than the control. In this series all but formula 12 had this identical effect.

Formulas 9 and 11 showed a more prolonged return of the blood sugar to its original level than the others. The later base held the

* Glyco Products, Inc., New York City.



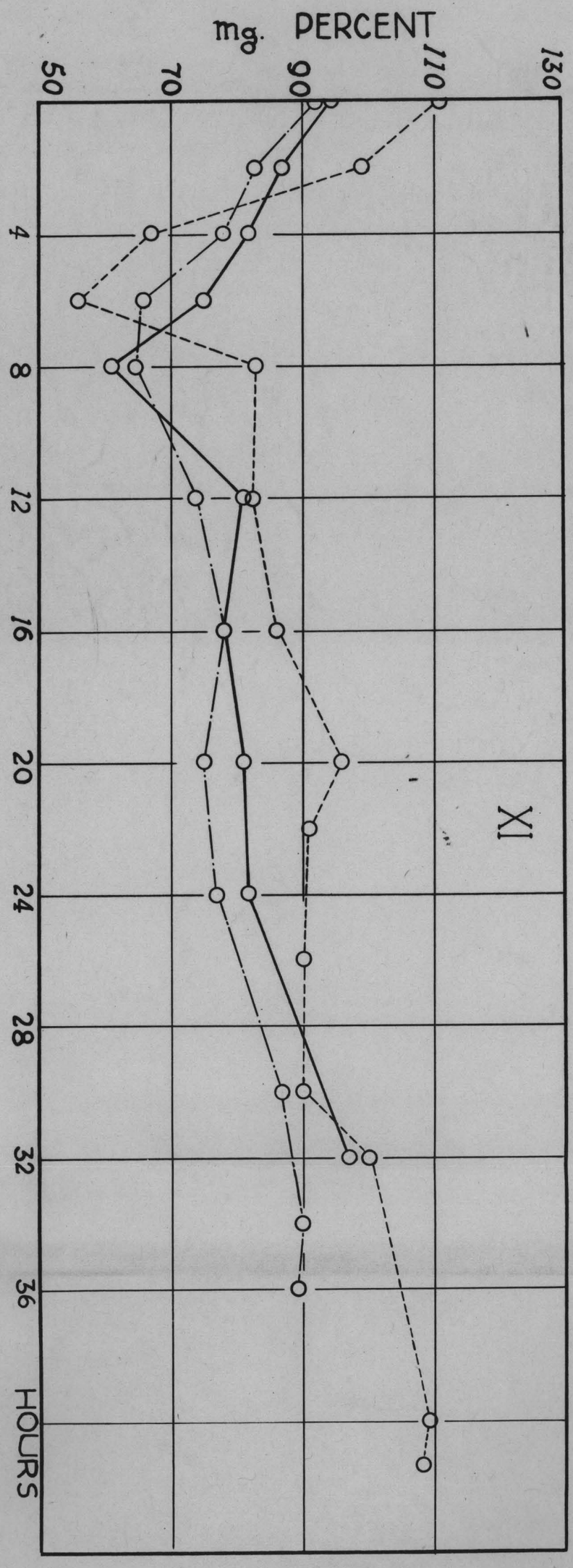
Glyceryl Tristearate
Anhydrous
Zinc-Insulin Crystals
1 Mg.



Glyceryl Tristearate
5 p.c. Water

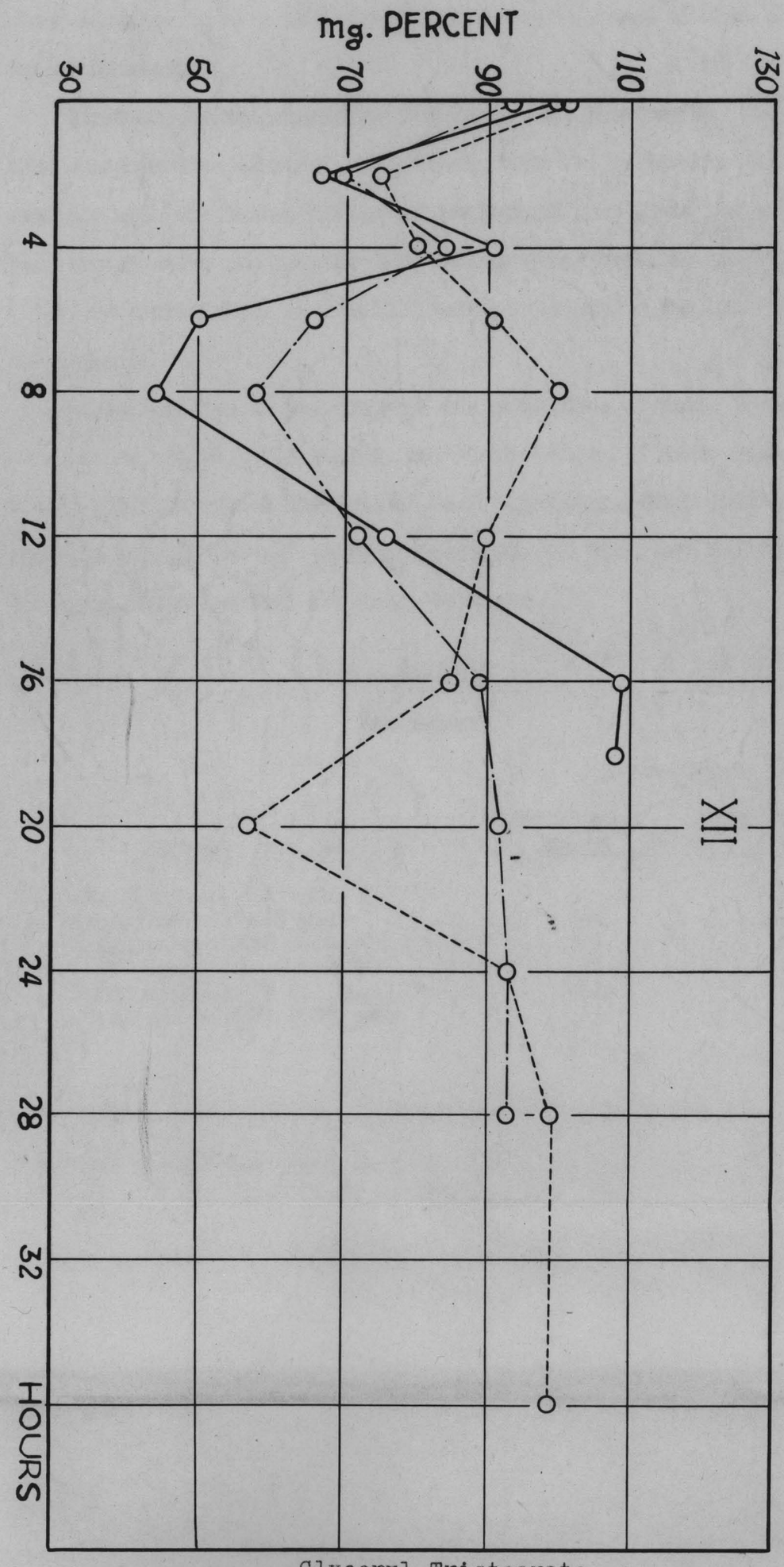
Zinc-Insulin Crystals

1 mg.



Glyceryl Tristearate
10 p.c. Water

Zinc-Insulin Crystals
1 mg.



Glyceryl Tristearate
20 p.c. Water
Zinc-Insulin Crystals
1 mg.

blood sugar value at a lower level for a longer period of time than did the other bases.

Extremely uneven absorption was shown from formula 12. Perhaps this concentration of water, 20 percent, made the preparation too miscible with the tissue fluids and absorption took place too rapidly. Ten percent water was probably the optimum concentration, and allowed a more even absorption of insulin from the surface to the center of the implant.

Another factor in the study of the absorption of these preparations, as with the monostearate series, was the formation of dense connective tissue capsules around the implants and injections. This reaction probably had some effect on the absorption, but it is not possible to say at this time what its exact role was.

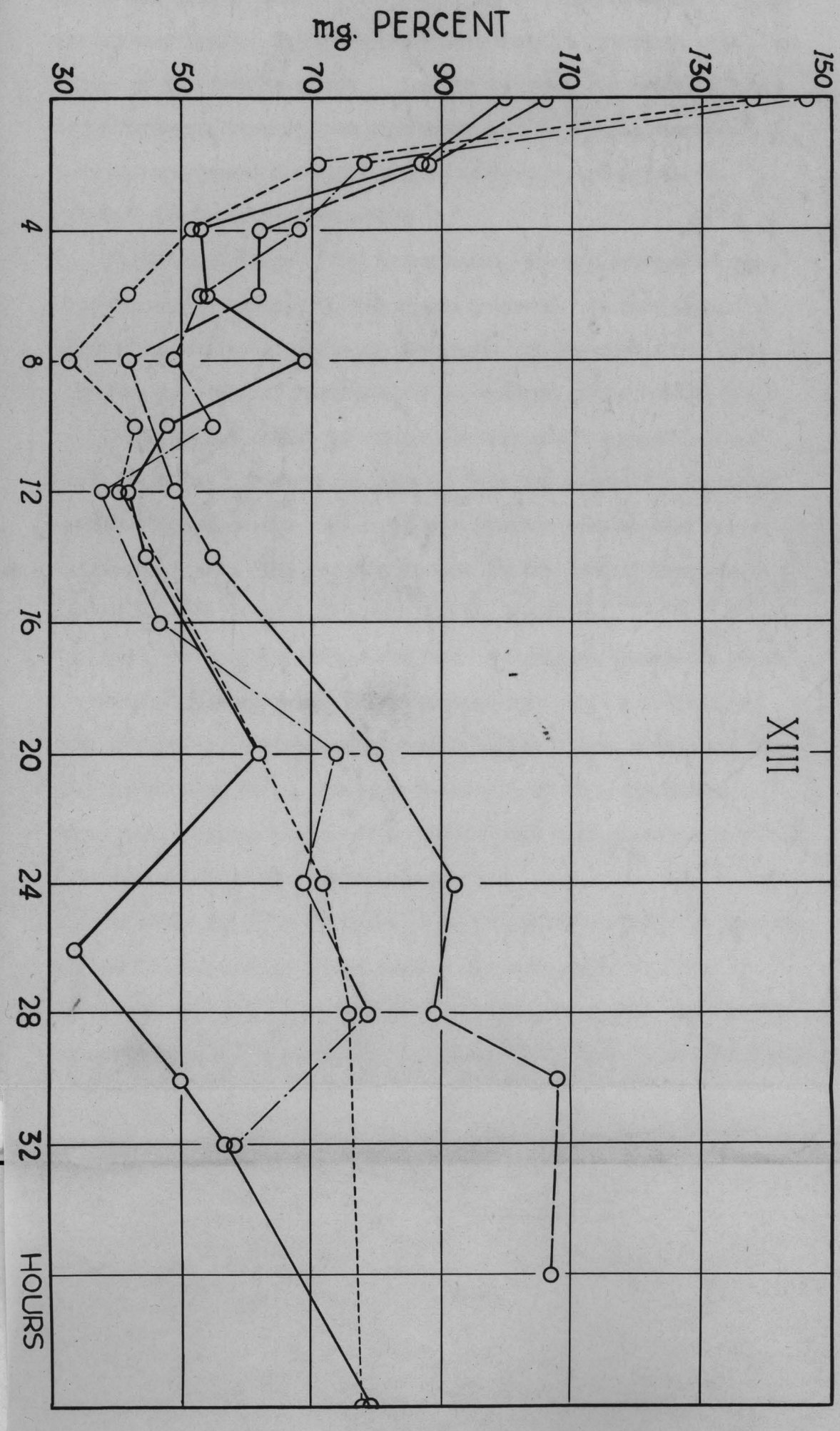
Experiment 4

Petrolatum

Formula	Implantation: Initial Drop hours	Implantation: Length of action hours
13. Petrolatum - 0.1000 gm. Zn-insulin - 0.0003 gm.	2-4	30-40
(a) coated with wool fat	2-4	---
(b) coated with paraffin	--	---
(c) coated with G.M.S.	6-12	50
(d) coated with collodion	--	---

Fifteen experiments were carried out with preparation 13, and 4 typical results are shown on curve XIII.

From these results it was concluded that petrolatum exerted a beneficial effect in prolonging the absorption of insulin. Only 2 of



Petrolatum

Zinc-Insulin Crystals

0.3 mg.

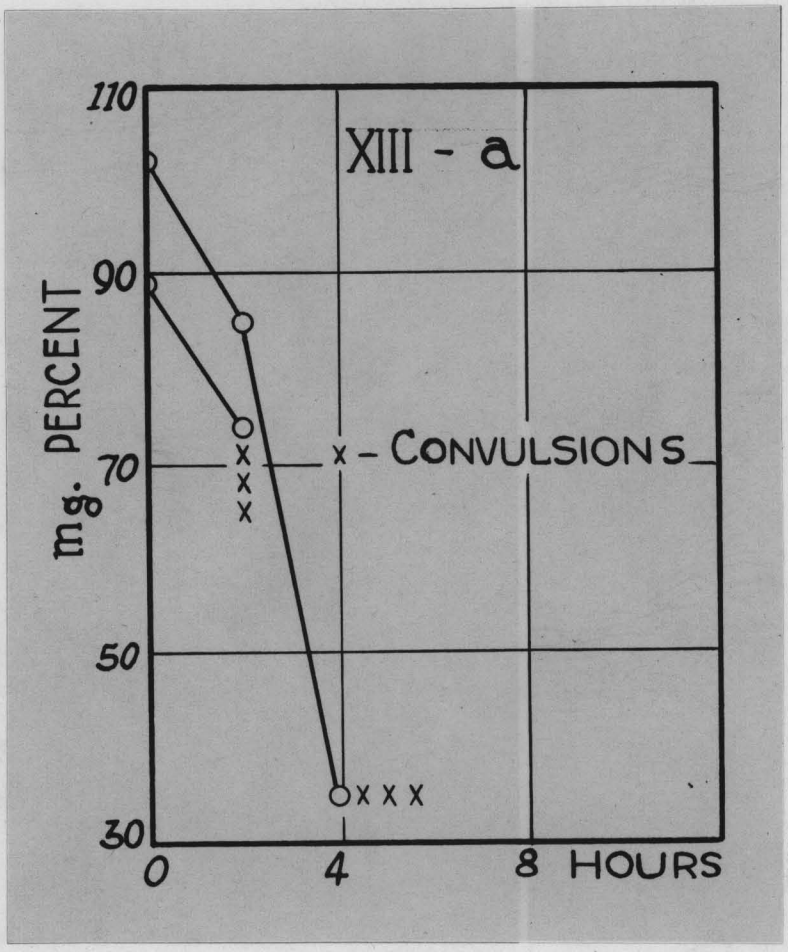
15 experiments returned to the original level and these were after 30 hours duration. Five of these experiments were continued 75 hours. During the last 35 hours, the blood sugar remained at approximately its 40 hour level. It was impossible to conclude at which point the effect of the insulin stopped. Any figure below the original level up to 34 hours, however, was attributed to the insulin, because in several experiments the blood sugar was found to return to its original level during this period.

At the conclusion of the experiments, the implanted area was examined and no capsule formation was observed. In most cases the preparation appeared completely absorbed. In those in which some remained, crystals of insulin could be observed under a microscope.

An objection in the petrolatum results was the rapid descent from normal in the first few hours. This was supposedly from the surface of the implant. Attempts were made to prevent this by coating the preparations. The preparation used in the coating experiments was the same as formula 13 except for the coating.

Wool fat contains cholesterol and cholesterol esters and forms a water-in-oil emulsions. It was thought that with a coating of this material an emulsion might result in the tissue fluids and slow the initial absorption. The results are shown on curve XIII-a. These were disappointing and the rabbits went into convulsions within 2 to 4 hours after the implantation.

Paraffin was then attempted as a coating in an effort to prolong the initial drop of the blood sugar. The results of 2 of the experiments are shown on curve XIII-b. Six experiments were carried out and all showed that little or no absorption took place from these

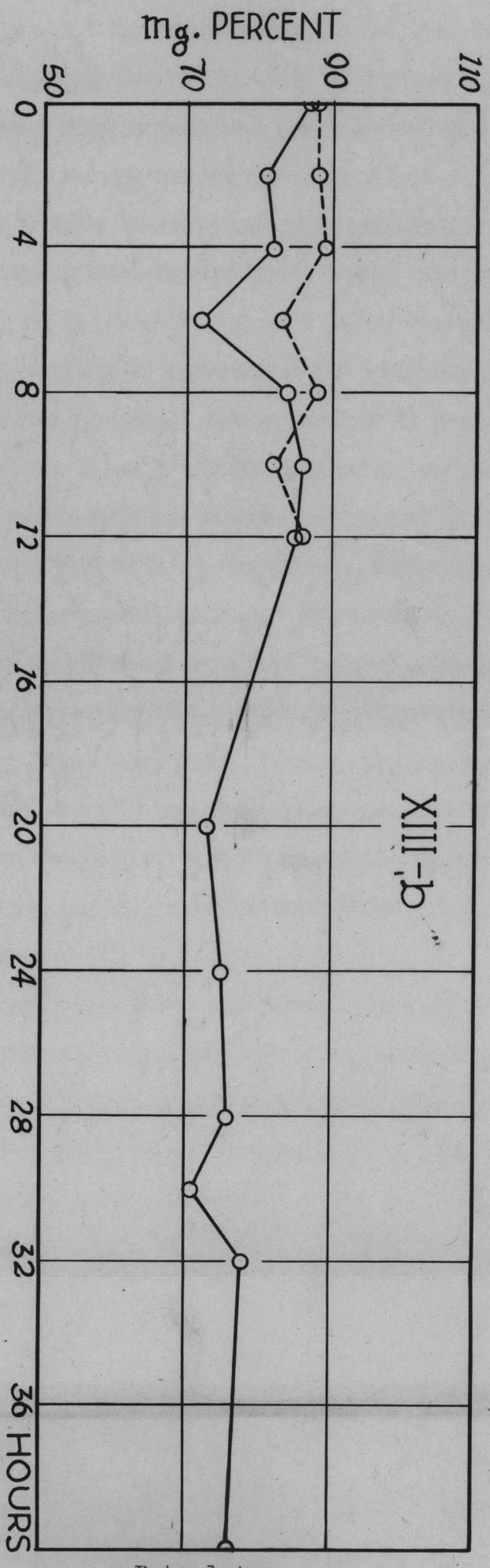


Petrolatum

Zinc-Insulin Crystals

0.3 mg.

Coated with Wool Fat



Petrolatum
Zinc-Insulin Crystals
0.3 mg.

Coated with Paraffin

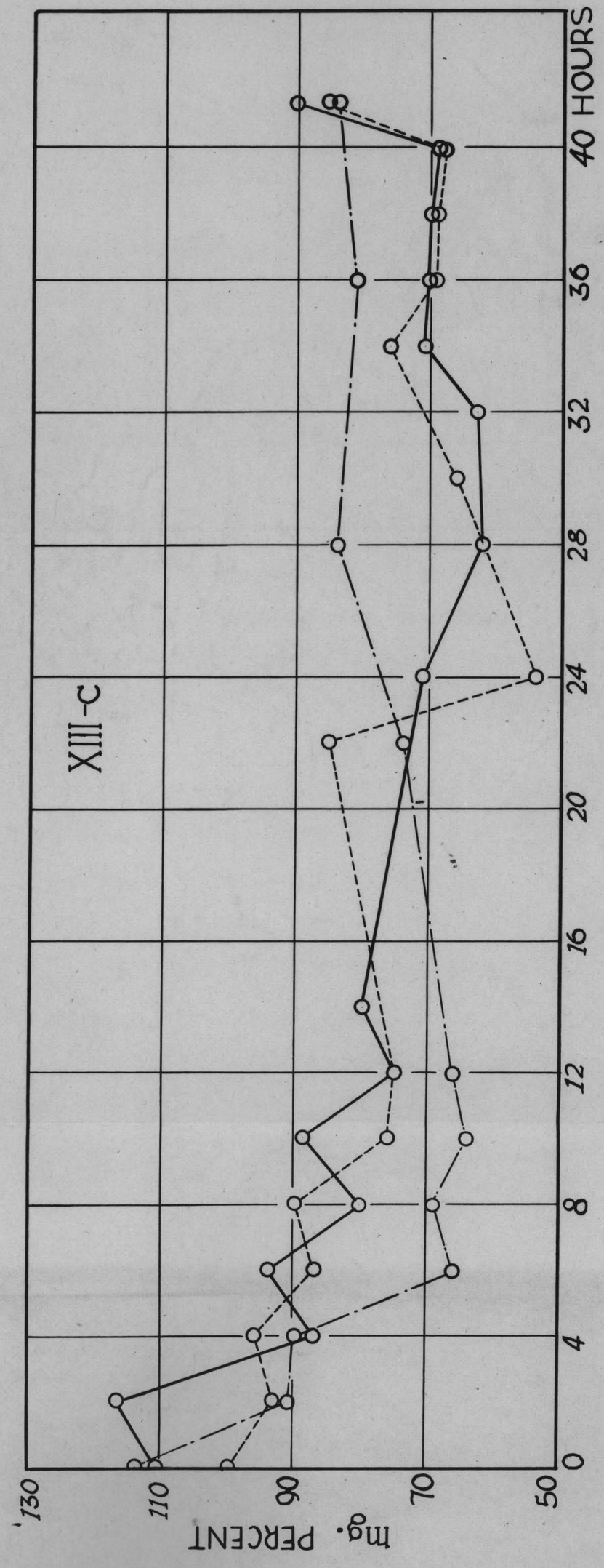
coated implants. No capsule formation was observed.

Glyceryl monostearate was also used to coat the petrolatum preparation. Eight experiments were observed with this type of coating. The results are shown on curve XIII-c.

These results indicate that glyceryl monostearate, as a coating, prolonged the initial drop of blood sugar. The fall from normal was irregular, but 3 of the experiments showed that the lowest drop occurred from 24 to 28 hours after the implantation. Five experiments were continued 74 hours. During the last 24 hours the blood sugar remained at the level of the 50 hour determination. This indicated that this preparation was active for at least 50 hours. Three experiments resulted in no absorption. This was probably due to a too heavy coating of the glyceryl monostearate.

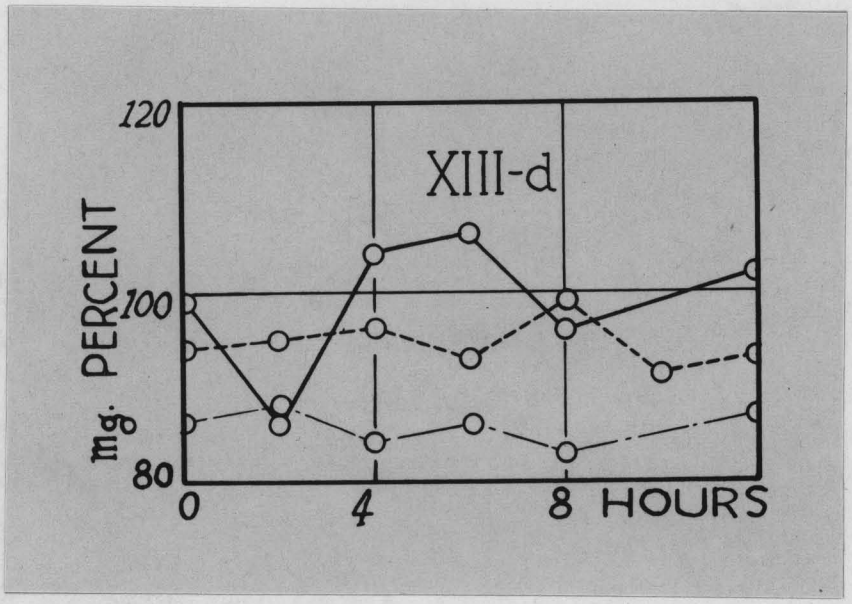
Collodion membranes were used to good advantage in prolonging the action of sex hormones. These membranes theoretically do not allow the passage of protein. No work was observed in the literature on this subject and 3 experiments were carried out in which collodion was used as the coating. These results are shown on curve XIII-d. The collodion coating prevented absorption and the implants were removed intact.

Glyceryl monostearate was the only coating of those attempted which exerted any beneficial effect in slowing the rapid initial lowering of the blood sugar. The type of preparation gave the best results in regard to slow and prolonged absorption, however, a heavy capsule formation resulted.



Petrolatum
Zinc-Insulin Crystals
0.3 mg.

Coated with Glyceryl Monostearate



Petrolatum

Zinc-Insulin Crystals
0.3 mg.

Coated with Collodion

Experiment 5

Aquaphor*

Formula	Implantation: Initial Drop hours	Implantation: Length of action hours
14. Aquaphor------ 0.1000 gm. Zn-Insulin--- 0.0003 gm.	6-8	12-14
15. Aquaphor------ 0.0950 gm. Water (5%)--- 0.0050 gm. Zn-Insulin--- 0.0003 gm.	4	---
16. Aquaphor------ 0.0900 gm. Water (10%)-- 0.0100 gm. Zn-Insulin--- 0.0003 gm.	8	12-16
17. Aquaphor------ 0.0800 gm. Water (20%)-- 0.0200 gm. Zn-Insulin--- 0.0003 gm.	8	12-14

A series of experiments was carried out using 0.3 mg., 7.2 units, of zinc-insulin crystals incorporated with this preparation containing varying percentages of water, formulas 14, 15, 16 and 17. The results of 3 experiments of each group are shown on curves XIV, XV, XVI and XVII.

This data indicates that the duration of action was no longer than that of the control. The initial drop from the original level was slower than that of the control, but there was a rapid return to the original level.

Unexplained ~~was~~ were the results from the preparation, formula 15, where all 3 rabbits went into convulsions. Two of 4 experiments with formula 17 ended in convulsions. None of the other experiments showed any convulsions.

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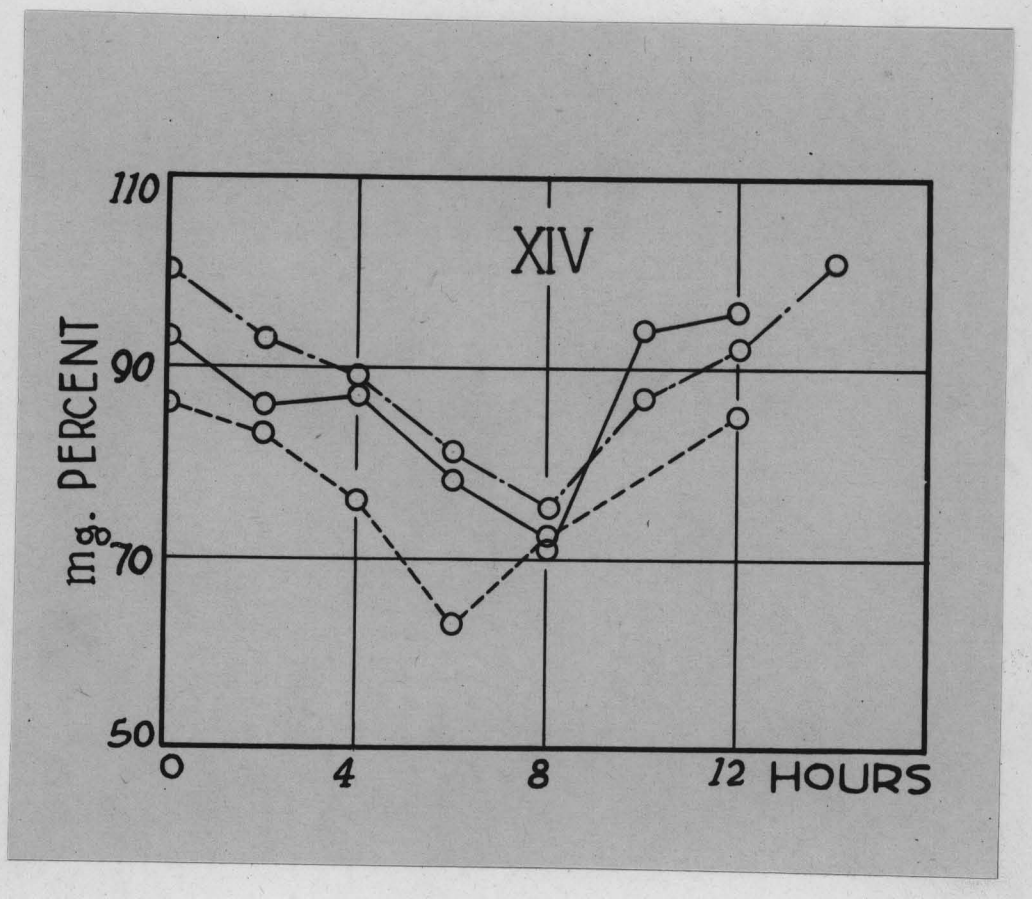
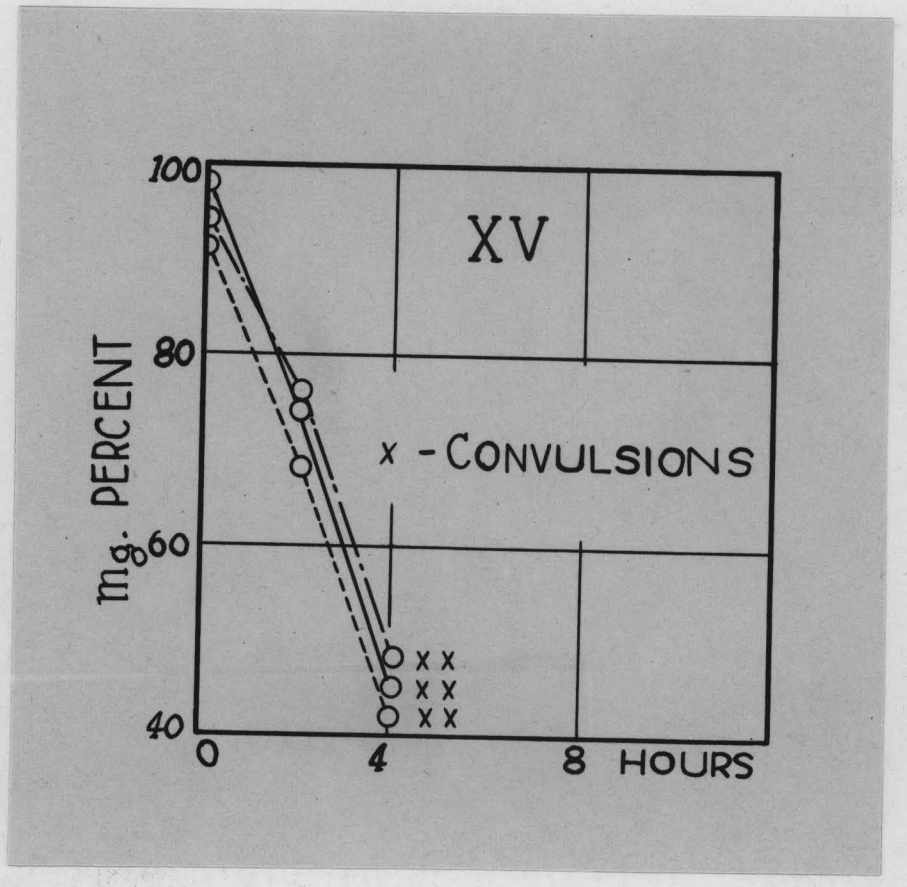
* Duke Laboratories, Inc., Stanford, Conn., "6% Cholesterol esters in a hydrocarbon base".



Aquaphor

Zinc-Insulin Crystals
0.3 mg.

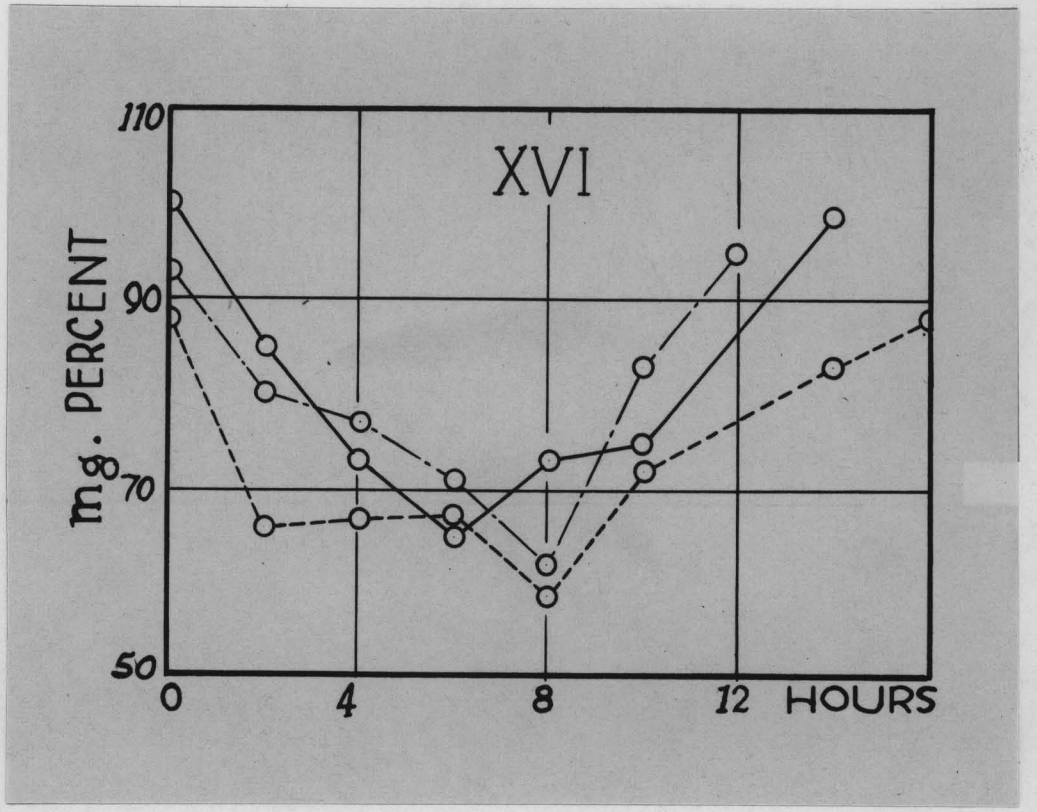


Aquaphor

5 p.c. Water

Zinc-Insulin Crystals

0.3 mg.

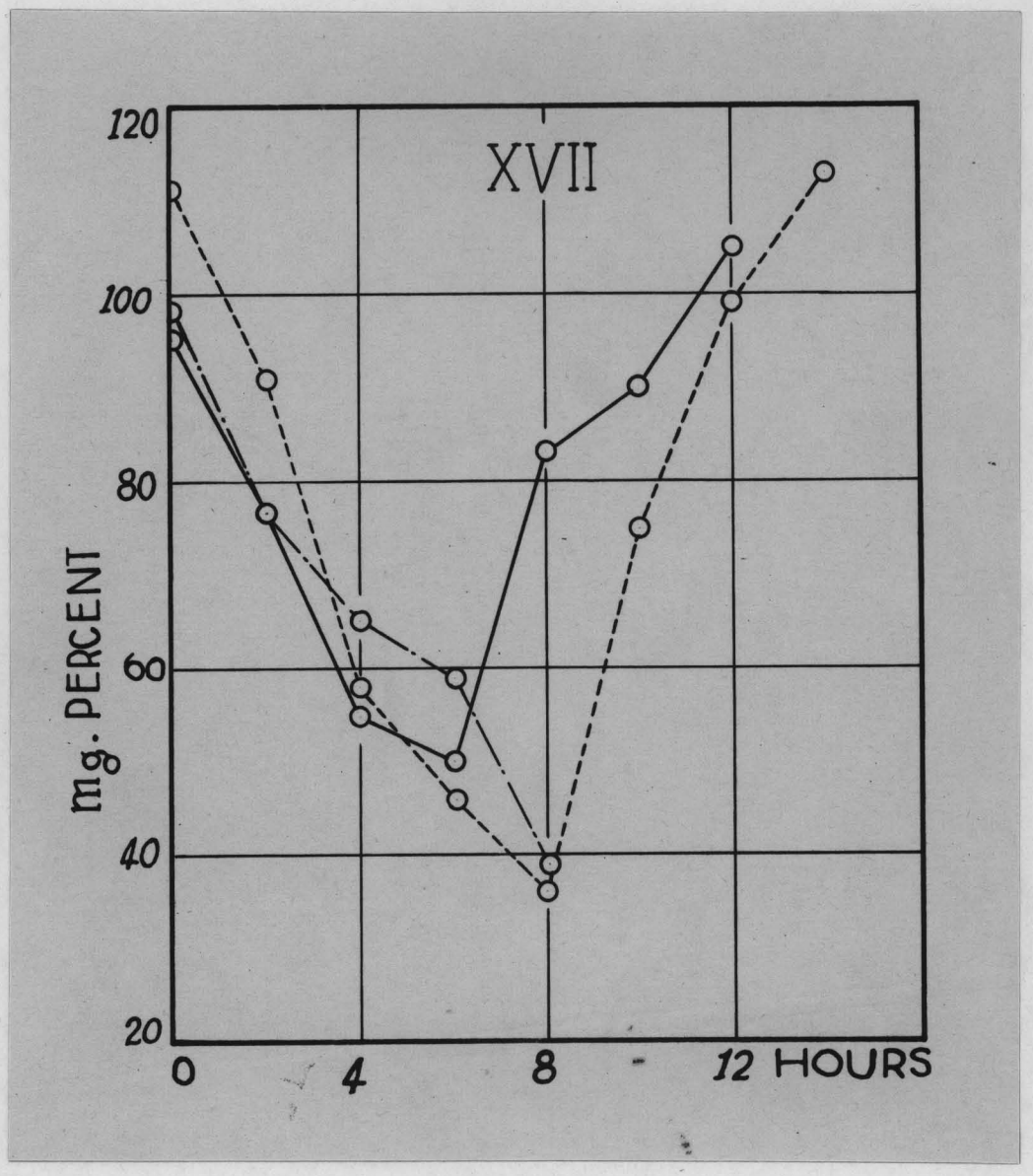


Aquaphor

10 p.c. Water

Zinc-Insulin Crystals

0.3 mg.



Aquaphor
20 p.c. Water
Zinc-Insulin Crystals
0.3 mg.

Experiment 6

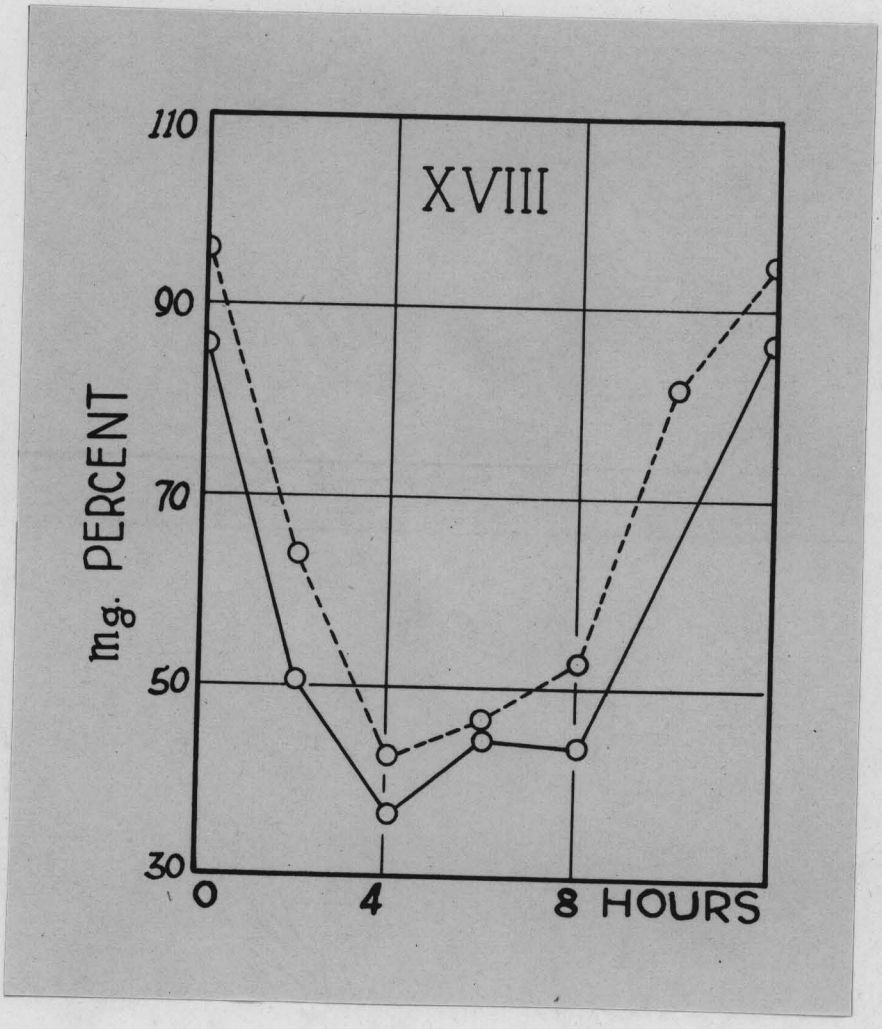
Miscellaneous

Formula	Initial Drop hours	Length of action hours
18. Petrolatum ---0.0750 gm. Paraffin------0.0250 gm. Zn-Insulin----0.0003 gm.	4	12
19. Petrolatum (26.5%) Aerosol (0.5%)---1.0 gm. Cetyl Alcoh(8.0%) Insulin U-20---------1.0 cc.	2	8-12
20. Petrolatum (26.5%) Aerosol (0.5%) - 2.0 gm. Cetyl Alcohol (8.0%) Glyc. Tannic Acid------ 0.5 cc. Insulin U-20---------- 0.5 cc.	6	30-40
21. Cod Liver Oil 6.0 gm. White Beeswax 1.0 gm.---0.1000 gm. Zn-insulin crystals------0.0003 gm.	6	14

The results of the absorption of formula 18 are shown on curve XVIII. This curve indicates that the presence of 25 percent paraffin caused the petrolatum to lose its property of prolonging the absorption. This preparation did not have any capsule surrounding it at the conclusion of the experiment.

Standard insulin solution was used in formula 19 and it was injected subcutaneously by means of the modified syringe so that each rabbit received 5 units of insulin. The results are shown on curve XIX.

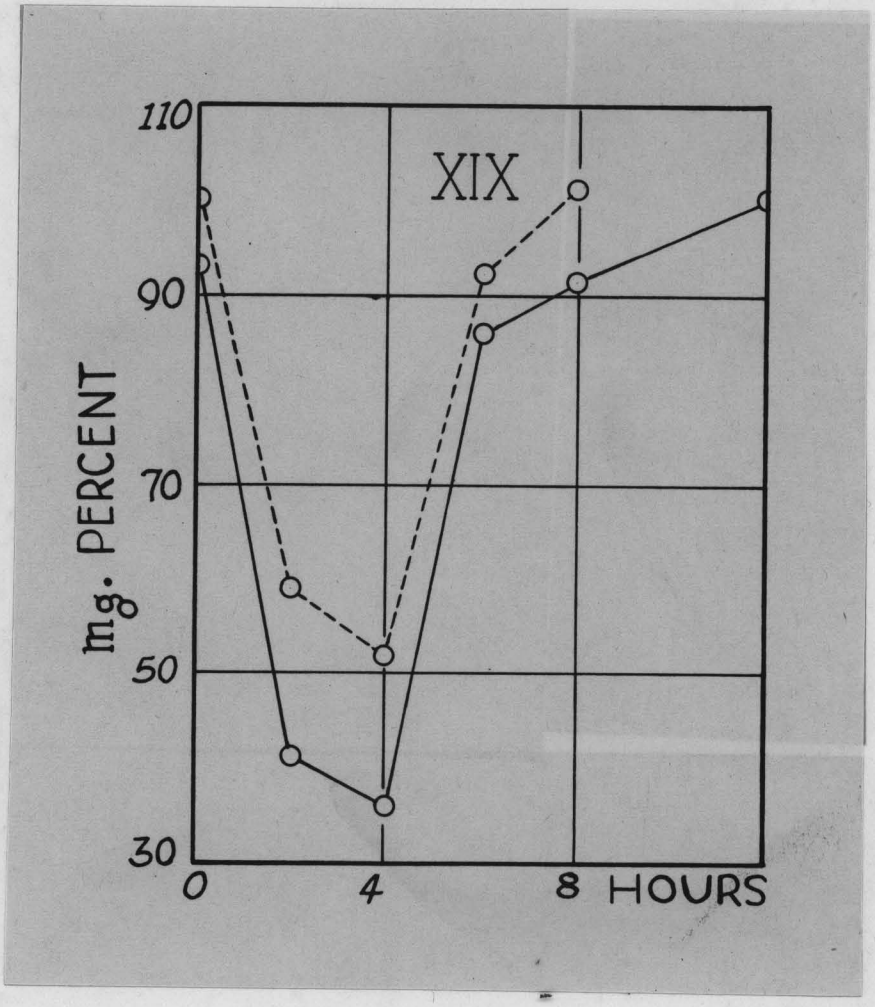
When glycerite of tannic acid was added to this preparation, formula 20, the absorption took place at a much slower rate than the solution of insulin alone. The injected material was heavily encapsulated and the skin was discolored over the area of the injection.



Petrolatum

Paraffin

Zinc-Insulin Crystals
0.3 mg.



Petrolatum

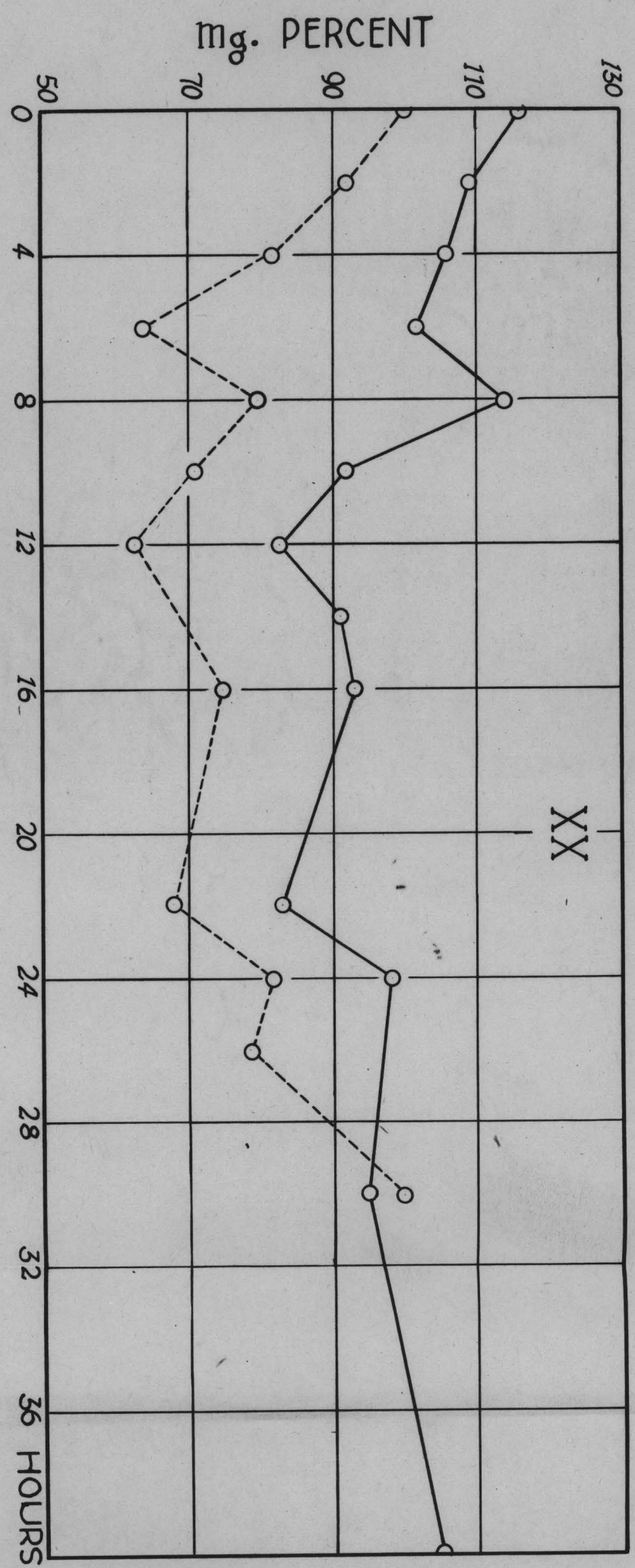
Aerosol

Cetyl Alcohol

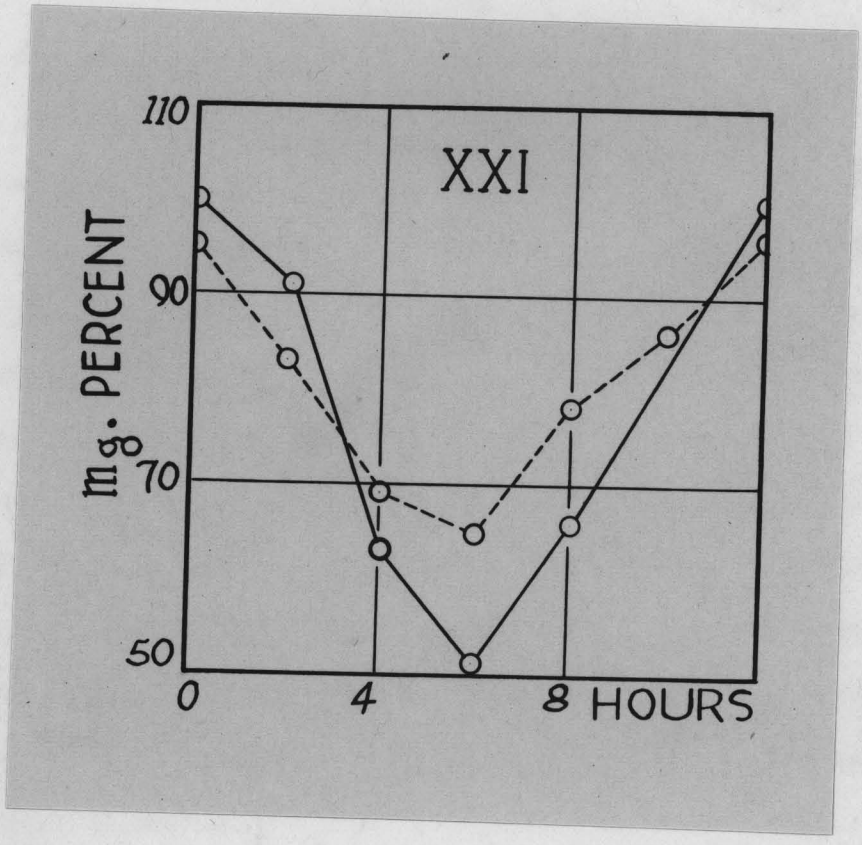
Standard Insulin Solution
5 Units

The results from this experiment are shown on curve XX.

Formula 21 did not exert any prolonging effect on the absorption of the insulin. The absorption was even and showed a duration of action which was similar to the control of zinc-insulin crystals. These results are on curve XXI.



Petrolatum--Aerosol--Cetyl Alcohol
Glycerite of Tannic Acid
Standard Insulin Solution
5 Units



Cod Liver Oil
White Beeswax
Zinc-Insulin Crystals
0.3 mg.

EXPERIMENTAL PART

CUTANEOUS

Reports in the literature, although contradictory, indicated that insulin might be absorbed through the skin. Attempts were made to study the cutaneous absorption of insulin. Rabbits, weighing between 1.5 and 2.0 kg., were used as test animals. Between experiments they were kept on a diet of hay and oats, but were not fed 24 hours previous to the experiments and received no food during the experiments. In these experiments the hair of the abdomen was removed with an electric clipper. No vehicle was used for the standard solutions of insulin which were applied by inunction. Experiments were carried out using 20, 40, and 160 units of insulin. None produced a marked lowering of the rabbits' blood sugar. Blood sugar analysis was carried out by Folin's Micro-Method.[1] Results from this series are shown in Tables 1, 2, and 3.

Table 1

20 units

Hours	Time	Mg. P.c. Glucose
	8:30 A.M. normal.....	98.02
	8:40 A.M. applied 20-U	
1	9:40 "	96.35
2	10:40 "	97.41
3	11:40 "	97.17
4	12:40 P.M.	98.56

Table 2

40 units

Hours	Time		Mg. p.c. Glucose
	8:05 A.M.	normal.....	95.10
	8:15 "	Applied 40-U	
1	9:15 "	.....	93.33
2	10:15 "	.....	92.52
3	11:15 "	.....	96.78
4	12:15 P.M.	.....	95.26

Table 3

160 units

Hours	Time		Mg. p.c. Glucose
	8:50 A.M.	normal.....	93.02
	9:30 "	Applied 160-U	
1	10:30 "	.....	97.56
2	11:30 "	.....	95.24
3	12:30 P.M.	.....	95.71
4	1:30 "	.....	96.50
5	2:30 "	.....	93.89

This data indicated that insulin was not absorbed through the skin in doses as high as 160 units.

Starkenstein and Hendrych[2] reported that insulin applied to the skin produced a marked lowering of the blood sugar, if applied after rubbing the skin with petroleum ether or chloroform, both solvents for cholesterol. They postulated that cholesterol hindered absorption. They did not indicate in what form the insulin was applied.

A series of experiments was carried out using varying units of

standard insulin solutions applied to the skin of rabbits after pretreating it with chloroform, petroleum ether, and ethyl alcohol. Since no reports were observed in the literature showing the quantitative solubility of cholesterol in these solvents, it was thought to the point to carry out such an experiment. The results are shown in Table 4.

Table 4

Solubility of Cholesterol (22 °C.)

In Chloroform.....	0.4819 gm. per cc.
In Petroleum ether....	0.0133 gm. per cc.
In Ethyl Alcohol.(95%)	0.0084 gm. per cc.

The results of the absorption of insulin applied to the skin after pretreatment with chloroform are shown in Tables 5, 6, 7 and 8, and on Curve XXII.

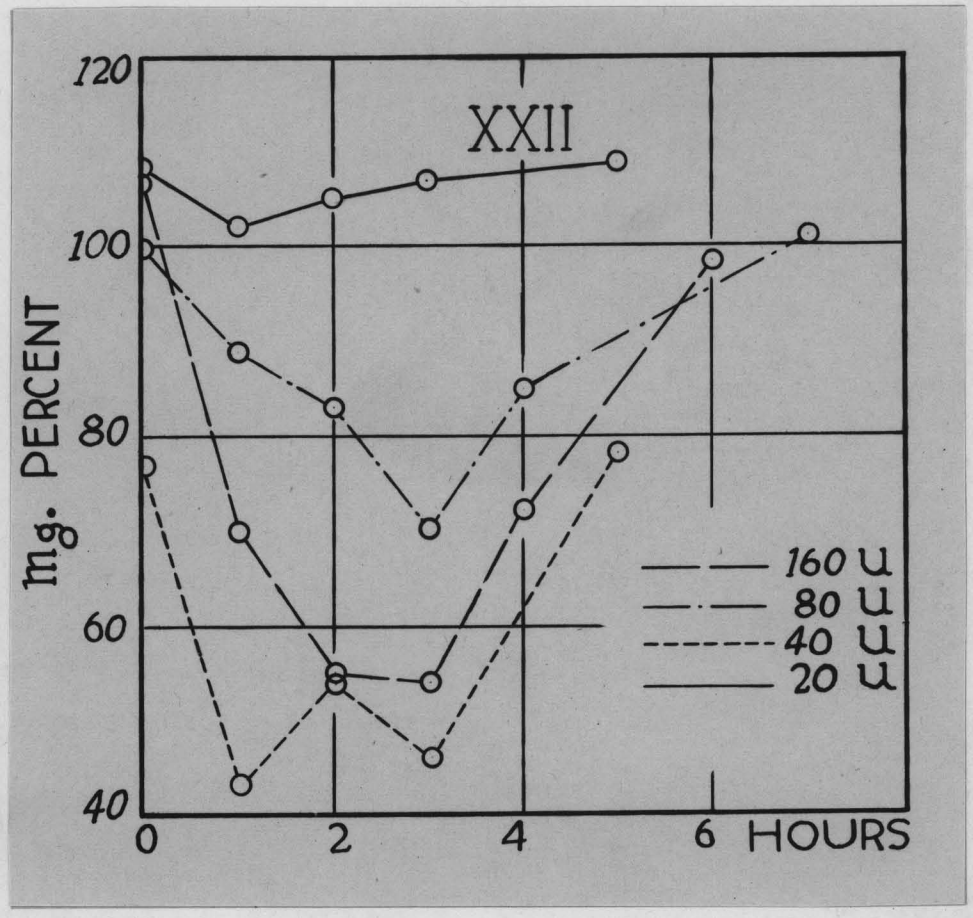
Table 5

20 units

Chloroform Pretreatment

A.

Hours	Time	Mg. D.C. Glucose
	8:15 A.M. normal.....	91.32
	8:25-8:30 Applied $CHCl_3$	
	8:30-8:35 Applied 20-U	
1	9:35 A.M.	90.09
2	10:35 "	79.06
3	11:35 "	90.90
5	1:35 P.M.	99.99



Absorption of Insulin applied to the skin after pretreatment with chloroform

Table 5

20 units

Chloroform Pretreatment

B.

Hours	Time	Mg. p.c. Glucose
	9:35 A.M. normal.....	105.26
	9:50-9:55 Applied $CHCl_3$	
	9:55-10:00 Applied 20-U	
1	11:00 A.M.	104.16
2	12:00 Noon	93.45
3	1:00 P.M.	99.00
4	2:00 "	109.28
5	3:00 "	106.33

C.

Hours	Time	Mg. p.c. Glucose
	9:25 A.M. normal.....	108.49
	9:50-9:55 Applied $CHCl_3$	
	9:55-10:00 Applied 20-U	
1	11:00 A.M.	102.56
2	12:00 Noon	93.45
3	1:00 P.M.	106.62
4	2:00 "	---
5	3:00 "	109.09

Table 6

40 units

Chloroform Pretreatment

A.

Hours	Time	Mg. p.c. Glucose
	10:20 A.M. normal.....	68.96
	10:45-10:50 Applied $CHCl_3$	
	10:50-11:00 Applied 40-U	
1	12:00 Noon	43.10
2	1:00 P.M.	33.33
3	2:00 "	47.61
5	4:00 "	60.60

B.

Hours	Time	Mg. p.c. Glucose
	1:45 P.M. normal.....	76.66
	2:25-2:30 Applied $CHCl_3$	
	2:30-2:35 Applied 40-U	
1	3:35 P.M.	43.10
2	4:35 "	54.94
3	5:35 "	46.51
5	7:35 "	78.10

C.

Hours	Time	Mg. p.c. Glucose
	8:20 A.M. normal.....	83.33
	8:30-8:35 Applied $CHCl_3$	
	8:35-8:40 Applied 40-U	
1	9:40 A.M.	47.61
2	10:40 ".	36.49
	11:40-3:40 Rabbit was prostrate- unable to get blood sample	
7¼	4:00 P.M. Convulsions	

Table 6

40 units

Chloroform Pretreatment

D.

Hours	Time	Mg. n.c. Glucose
	2:05 P.M. normal.....	95.69
	2:35-2:40 Applied $CHCl_3$	
	2:40-2:45 Applied 40-U	
1	3:45 P.M.	95.69
2	4:45 "	95.69
4	6:45 "	105.26

E.

Hours	Time	Mg. n.c. Glucose
	8:55 A.M. normal.....	98.03
	9:00-9:05 Applied $CHCl_3$	
	9:05-9:15 Applied 40-U	
1	10:15 A.M.	84.74
2	11:15 "	83.33
3	12:15 "	95.24
4	1:15 "	88.11

Table 7

80 units

Chloroform Pretreatment

Hours	Time	Mg. n.c. Glucose
	1:00 P.M. normal.....	100.00
	1:10-1:15 Applied $CHCl_3$	
	1:15-1:20 Applied 80-U	
1	2:20 P.M.	89.65
2	3:20 "	83.33
3	4:20 "	70.43
4	5:20 "	85.59
7	8:20 "	101.09

Table 8

160 units

Chloroform Pretreatment

A.

Hours	Time	Mg. n.c. Glucose
	1:00 P.M. normal.....	95.69
	1:15-1:20 Applied $CHCl_3$	
	1:20-1:30 Applied 160-U	
1	2:30 P.M.	74.84
2	3:30 "	65.51
3	4:30 "	71.43
6	7:30 "	94.44

B.

Hours	Time	Mg. n.c. Glucose
	12:55 P.M. normal.....	95.23
	1:00-1:05 Applied $CHCl_3$	
	1:05-1:15 Applied 160-U	
1	2:15 P.M.	66.66
2	3:15 "	74.07
3	4:15 "	64.51
4	5:15 "	45.45
6	7:15 "	45.45
	Rabbit went into convulsions and was treated with glucose intravenously.	

C.

Hours	Time	Mg. n.c. Glucose
	12:45 P.M. normal.....	108.11
	1:00-1:05 Applied $CHCl_3$	
	1:05-1:15 Applied 160-U	
1	2:15 P.M.	69.68
2	3:15 "	55.55
3	4:15 "	54.94
4	5:15 "	72.20
6	7:15 "	95.79

This data indicates that it was possible to lower the blood sugar level by applying standard insulin solutions to the abdomen of rabbits which was pretreated with chloroform. Two of the 11 rabbits in this series went into convulsions. There was a marked lowering of the blood sugar with the application of 80 and 160 units. Little or no lowering was observed with 20 units and 2 experiments with 40 units failed to show a marked drop in the blood sugar level.

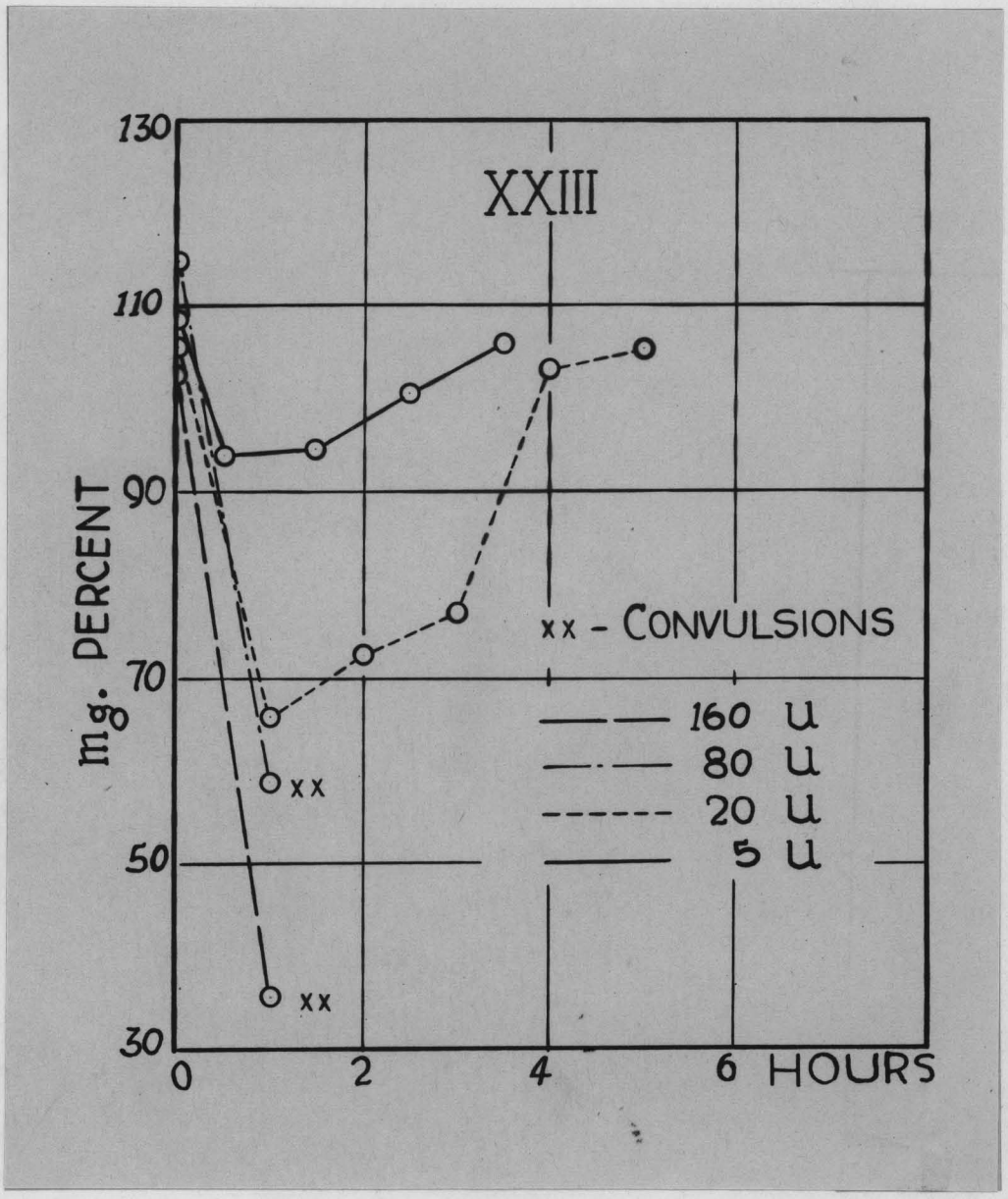
Petroleum ether was the next substance employed to pretreat the skin. The results from this series are shown in Tables 9, 10, 11, and 12, as well as on curve XXIII.

Table 9

5 units

Petroleum Ether Pretreatment

Hours	Time	Mg. p.c. Glucose
	9:15 A.M. normal.....	108.10
	10:10-10:15 Applied P.E.	
	10:15-10:20 Applied 5-U	
1/2	10:50 A.M.	93.02
1-1/2	11:50 "	94.34
2-1/2	12:50 P.M.	100.00
3-1/2	1:50 "	105.26



Absorption of insulin applied to the skin after pretreatment with petroleum ether

Table 10

20 units

Petroleum Ether Pretreatment

A.

Hours	Time	Mg. D.C. Glucose
	9:30 A.M. normal.....	105.26
	9:35-9:40 Applied P.E.	
	9:40-9:45 Applied 20-U	
1	10:45 A.M.	65.73
2	11:45 "	71.68
3	12:45 P.M.	76.33
4	1:45 "	102.56
5	2:45 "	104.61

B.

Hours	Time	Mg. D.C. Glucose
	8:05 A.M. normal.....	106.62
	8:20-8:25 Applied P.E.	
	8:25-8:30 Applied 20-U	
1	9:30 A.M.	85.23
2	10:20 "	87.33
3	11:20 "	90.90
5	1:20 "	108.49

C.

Hours	Time	Mg. D.C. Glucose
	9:10 A.M. normal.....	94.34
	9:20-9:25 Applied P.E.	
	9:25-9:30 Applied 20-U	
1	10:30 A.M.	75.47
2	11:30 "	93.02
3	12:30 P.M.	95.05
5	1:30 "	94.43

Table 11

80 units

Petroleum Ether Pretreatment

Hours	Time		Mg. p.c. Glucose
	9:00 A.M.	normal.....	114.28
	9:15-9:20	Applied P.E.	
	9:20-9:30	Applied 80-U	
1	10:30	.....	58.11
2	11:30	Rabbit went into convulsions.	

Table 12

160 units

Petroleum Ether Pretreatment

Hours	Time		Mg. p.c. Glucose
	1:30 P.M.	normal.....	102.56
	1:55-2:00	Applied P.E.	
	2:00-2:10	Applied 160-U	
1	3:00 P.M.	.....	35.08
1-3/4	3:45 P.M.	Rabbit went into convulsions.	

It was established that 20 units would show a pronounced drop in the blood sugar after the skin was pretreated with petroleum ether. It was then assumed that all units above 20 would go through the skin. One experiment was carried out using 80 units and one using 160 units. Both rabbits went into convulsions within 2 hours. These results indicated that petroleum ether was a more efficient agent in preparing the skin than was chloroform. These results did not seem to substantiate the theory proposed by Starkenstein and Hendrych[2] that cholesterol was the agent present in the skin which prevented absorption of substances through it. If cholesterol was responsible for hindering absorption of substances through the skin, then chloroform should be a more efficient agent in preparing the skin for the absorption of insulin than petroleum ether by virtue of its much greater solvent action on cholesterol than petroleum ether. See Table 4.

In the next series of experiments, ethyl alcohol (95 percent) was employed to pretreat the skin. The results are shown in Tables 13, 14, 15, and 16.

Table 13

20 units

Alcohol Pretreatment

Hours	Time	Mg. D.C. Glucose
	9:20 A.M. normal.....	84.74
	9:30-9:35 Applied Alcohol	
	9:35-9:40 Applied 20-U	
1	10:40 A.M.	86.20
2	11:40 A.M.	82.30
3	12:40 P.M.	87.33
5	2:40 P.M.	83.33

Table 14

40 units

Alcohol Pretreatment

Hours	Time	Mg. p.c. Glucose
	8:15 A.M. normal.....	102.56
	8:20-8:25 Applied alcohol	
	8:25-8:30 Applied 40-U	
1	9:30 A.M.	112.99
2	10:30 A.M.	112.36
3	11:30 A.M.	92.12
4	12:30 P.M.	93.02
6	2:30 P.M.	93.45

Table 15

80 units

Alcohol Pretreatment

Hours	Time	Mg. p.c. Glucose
	9:20 A.M. normal.....	111.73
	9:25-9:30 Applied alcohol	
	9:30-9:35 Applied 80-U	
1	10:35 A.M.	111.73
2	11:35 "	117.64
3	12:35 P.M.	115.60
4	1:35 "	112.97
5	2:35 "	112.35

Table 16

160 units

Alcohol Pretreatment

A.

Hours	Time	Mg. n.c. Glucose
	8:50 A.M. normal.....	114.28
	8:55-9:00 Applied alcohol	
	9:00-9:05 Applied 160-U	
1	10:05 A.M.	117.64
2	11:05 "	111.11
3	12:05 P.M.	111.11
4	1:05 "	108.10
5	2:05 "	115.26

B.

Hours	Time	Mg. n.c. Glucose
	8:25 A.M. normal.....	96.15
	8:30-8:35 Applied alcohol	
	8:55-9:00 Applied 160-U	
1	10:00 A.M.	95.24
2	11:00 A.M.	95.69
3	12:00 Noon	96.61
4	1:00 P.M.	95.24
5	2:00 "	94.34
7	4:00 "	94.78

This data indicates that ethyl alcohol, 95% percent, failed to exert a beneficial effect and did not allow the penetration of the insulin solutions.

The skin area pretreated with petroleum ether and chloroform became hard, stiff, and dry several days after the experiments. This condition did not improve during a period of 2 weeks and the rabbits were killed. The skin area pretreated with alcohol was not changed to any great degree and after several days appeared normal.

APPLICATION IN OINTMENT VEHICLES

Starkenstein and Hendrych[2] reported than 20 units of insulin incorporated in a C-16 alcohol ointment produced a startling drop in a rabbit's blood sugar level if the skin was first prepared with petroleum ether. They did not disclose the formula of their ointment or the type of the insulin-- solution or solid.

A base was then prepared consisting of cetyl alcohol, 8 ~~percent~~ parts, aerosol, 0.5 ~~percent~~ part, and petrolatum, 26.5 ~~percent~~ parts. One cc. of a standard insulin solution containing 20 units per cc. was incorporated with 2 gm. of this base and applied to the shaved abdomen of a rabbit. This showed no effect and similar negative results were obtained with 40 units. This data is shown in Tables 17 and 18.

Table 17

20 units

Hours	Time	Mg. P.C. Glucose
	1:25 P.M. normal.....	90.90
	1:55-2:00 Applied P.E.	
	2:00-2:15 Applied Ointment-20-U	
1/4	2:30 P.M.	100.00
1	3:15 "	95.23
2	4:15 "	95.44
3	5:15 "	96.66

Table 18

40 units

Hours	Time	Mg. p.c. Glucose
	9:15 A.M. normal	88.49
	9:40-9:45 Applied P.E.	
	9:45-10:00 Applied 40-U	
1	11:00 A.M.	100.00
2	12:00 Noon	104.66
3	1:00 P.M.	98.03
4	2:00 P.M.	114.28

Two cc. of U-80 insulin solution, 160 units, was incorporated with 4 gm. of aquaphor and this preparation applied to the clipped abdomen of a rabbit pretreated with chloroform. The results of this are shown in Table 19.

Another preparation (160 units), was then rubbed on the abdomen of another rabbit pretreated with petroleum ether. These results are shown in Table 20. Neither experiment produced a lowering of the blood sugar.

Table 19

160 units

Chloroform Pretreatment

Hours	Time	Mg. p.c. Glucose
	12:45 P.M. normal	100.00
	12:50-12.55 Applied $CHCl_3$	
	12:55-1:05 Applied 160-U	
1	2:05 P.M.	103.90
2	3:05 "	98.10
3	4:05 "	101.01

Table 20

160 units

Petroleum Ether Pretreatment

Hours	Time	Mg. p.c. Glucose
	8:55 A.M. normal.....	109.05
	9:00-9:05 Applied P.E.	
	9:05-9:15 Applied 160-U	
1	10:15 A.M.	99.50
2	11:15 ".	98.79
4	1:15 P.M.	100.00
5	2:15 "	99.50

These results indicate that ointments hindered absorption of the insulin.

BIBLIOGRAPHY

EXPERIMENTAL PART

1. Folin, O., J. Biol. Chem., 77, 421, (1928).

2. Starkenstein, E. and Hendrych, F., Arch. f. exp. path. u. Pharmak., 182, 664, (1936).

CONCLUSIONS

SUBCUTANEOUS

1. The results indicated that petrolatum, per se, exerted the most beneficial effect in prolonging the action of the medicinal.
2. This property was destroyed by the addition of paraffin cholesterol esters (aquaphor), and cetyl alcohol and aerosol mixture.
3. The prolonging of the absorption was not entirely dependent upon the type of emulsion.
4. Preliminary experiments indicated that a water-in-oil type emulsion prolonged the absorption. Further work failed to confirm this indication. Commercial glyceryl tristearate, which results in a water-in-oil type emulsion, exerted a beneficial effect. On the other hand, aquaphor, which forms the same type of emulsion failed to extend the duration of action.
5. Preliminary work seemed to indicate that an oil-in-water type emulsion did not prolong the absorption. Additional results failed to confirm this indication. Commercial glyceryl monostearate, which forms an oil-in-water type emulsion, was shown to exert a beneficial effect in prolonging the action. The petrolatum, aerosol, and cetyl alcohol mixture, which also forms an oil-in-water type emulsion, did not exert this beneficial effect.

6. Experimental results indicated that 10 percent water was an optimum concentration for an even, prolonged absorption from those bases which had the ability to absorb water.
7. More absorption resulted from the preparations which contained the greatest amount of water than from those with less water. This was indicated by a lower drop in the blood sugar level.
8. The rapid descent of the blood sugar level from most of these bases during the first few hours was probably due to the absorption of the readily available insulin on the surface of the preparation. The maintaining of a low level might have been due to the absorption of insulin from within the base.
9. A capsule of connective tissue was found around all of the implants except those made with petrolatum. This suggested that this substance was of such consistency that it did not irritate the tissues and cause them to set up this defensive reaction to foreign bodies.
10. The encapsulated implants were removed at the conclusion of the experiments and extracted with water. These extracts were injected subcutaneously into untreated rabbits. No lowering of the blood sugar level was observed from these injections. This indicated that either the insulin was completely absorbed or that it was denatured.

CONCLUSIONS

CUTANEOUS

1. Standard insulin solutions, incorporated in ointment bases, failed to lower the blood sugar level after their application to the abdominal skin of rabbits either with pretreatment or without it. This indicated that ointments hindered absorption.
2. Standard insulin solutions, alone, in doses as high as 160 units failed to lower the blood sugar level after their application to the abdominal skin of rabbits.
3. It was found that if the skin was first treated with chloroform or petroleum ether, and standard solutions of insulin applied, a lowering of the blood sugar resulted.
4. This effect was not observed in experiments in which 95 percent ethyl alcohol was employed to treat the skin.
5. The skin of the rabbits became hard, dry, and stiff from the treatment with chloroform and petroleum ether, but not from the alcohol. This indicated that these solvents removed fatty material from the skin and allowed penetration to take place.
6. It was reported that cholesterol was the substance which prevented absorption. Since cholesterol was found to be more soluble in chloroform, the best absorption should be expected with the use of this substance. Such was not the case as the more pronounced effect resulted from the use of petroleum ether.

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