STUDIES ON THE MIGRATION OF GLUCOSE INTO THE RABBIT LENS *

by

Kenneth Merle Giles, B.S.

A THESIS

Presented to the Department of Biochemistry
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

December, 1958

*Part of the material presented in this thesis has been published[1].

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

ACKNOWLEDGEMENTS

The author is indebted to Dr. John E. Harris for his advice and support throughout the course of this study.

Sincere appreciation is expressed for the assistance and helpful suggestions of both Dr. Ellen Talman and Dr. John T. Van Bruggen during parts of this project.

Financial support was received from the Frank E. Ralston Student Assistantship Program and the National Council to Combat Blindness.

TABLE OF CONTENTS

LIST OF FIGURES

Figure 13. Radioelectrophoretograms of aqueous and aqueous plus carrier insulin following intravenous injection of I^{131} labeled insulin into normal rabbits.

Figure 14. Time course of activity considered to be insulin in the plasma and aqueous of normal rabbits following the intravenous administration of I^{131} labeled insulin.

Figure 15. Time course of activity considered to be insulin in the plasma and aqueous of diabetic rabbits following the intravenous administration of I^{131} labeled insulin.

Figure 16. Time course of activity considered to be insulin in the plasma and aqueous of rabbits with exogenous hyperglycemia following the intravenous administration of I^{131} labeled insulin.

Figure 17. Composite of activity considered to be insulin in the plasma of rabbits under varied conditions following the intravenous administration of I^{131} labeled insulin.

Figure 18. Composite of activity considered to be insulin in the aqueous of rabbits under varied conditions following the intravenous administration of I^{131} labeled insulin.

Figure 19. Comparison of the radioelectrophoretic patterns of plasma from rabbits under varied conditions drawn 15 minutes following the intravenous administration of I^{131} labeled insulin.

LIST OF TABLES

I. INTRODUCTION

The lens is one of the many tissues whose function is adversely influenced by alterations in its normal metabolic scheme. Cataracts resulting in loss of vision are one of the more obvious manifestations of these aberrations. However, studies on the lens are not limited in value to the ophthalmologist alone but they may point to answers to problems in other fields as well. Since one of the common malfunctions of the lens occurs in the diabetic state, considerable interest has been focused on the various aspects of lens metabolism related to this disease. Although the effect of diabetes, insulin and associated subjects has been extensively investigated many problems still remain unanswered. This thesis is submitted as a continuation of these studies.

Because of its importance to proper vision the lens has long been of interest to both scientists and non-scientists. As early as the tenth century a rather complete text on the eye and its diseases was written by Ali ibn Isa el-Kahhal of Baghdad.(2) His published thoughts included a mixture of Arabic and Galenic ideas concerning the nature of the eye. The lens was described as the organ by which vision was actually accomplished, both directing the flow of and receiving the return of the 'visual spirit'. Even as early as this the nutritional supply to the lens was under consideration. The nutrient supply to the lens was thought to be "furnished by the vitreous which is placed close to it, one behind the other in accordance with the providence of God". This is now known not to be entirely true.

The lens arises from ectodermal tissue in direct contact with the neural ectoderm of the optic outgrowth. Since it arises from only one germ layer and is composed of only one type of cell it is different from many other tissues where a mixture of cell types is encountered. As a result the lens provides a single cell type in various stages of development for study.

The lens plate appears at about the third week of intrauterine life. By the fourth week the plate has invaginated to form the lens vesicle. Following this the vesicle separates from the surface and is filled by the growth of lens fibers from the posterior surface. The anterior cells remain as simple cuboidal epithelium. In later life the lens growth is primarily from cells located at the equator. As the lens continues to grow the older cells are not desquamated as is the case with other tissues such as the skin. Rather the older more inactive tissue is continually pushed to the center of the increasing lens mass.

The lens is completely surrounded by a non-cellular laminated membrane secreted by the lens epithelium. It is a tough elastic membrane which might grossly be compared to a plastic bag enclosing the mass of lens tissue. Anteriorly this lens capsule is thicker than it is at the posterior surface. Directly beneath the anterior capsule are the cuboidal epithelial cells of the lens. When the capsule is stripped from the lens these cells are also removed. Posteriorly no epithelial cells are present and the capsule is in direct contact with the lens fibers.

This capsule across which all nutrients and metabolic products must pass in the intact condition was recently studied by Pirie (3).

The ox capsules studied contained fourteen per cent nitrogen, ten per cent carbohydrate, nine per cent reducing sugar after hydrolysis, and about one per cent hexosamine. No phosphorus was present. She felt that the protein component had many characterists of collagen but was not identical with collagen.

The lens is an avascular structure which depends on the surrounding tissues and fluids for its nutritional supply. In this manner the lens may be likened to a single cell, the capsule serving as the cell membrane. It maintains a high K:Na ratio[4] as do other cells. Because of its avascular nature the lens may be removed intact with a minimum of trauma providing an experimental tissue only slightly altered from its normal state.

The gel-like vitreous humor is in contact with the posterior surface of the lens and plays only a minor role in supplying nutrients to it. The anterior surface is bathed by the aqueous humor which is replaced at a rate of about one per cent per minute. It is reasonable to expect that metabolic process will be more rapid anteriorly simply because of the more ready supply of nutrients and more efficient means of disposing of the waste products.

Although the total energy requirement of the lens is relatively low as compared to most other tissues there is a real need for a continuing energy supply to the lens to maintain its several functions. Probably the most important immediate need of a stable energy supply is for the maintenance of the transparency of the lens without which proper vision would be impossible. The loss in transparency is one of the early signs of a failing lenticular metabolism whether it is induced by traumatic handling of the lens, lack of sufficient energy

supply, poisoning of enzyme systems[5] or by other means[6, 7]. Energy is also needed for proper growth and development with increasing age and for the maintenance of the proper elasticity required for accomodation.

The amount of oxygen presented to the lens is limited to the amount physically dissolved in the aqueous humor. Thus the lens would be expected to have a rather sluggish respiratory system as compared to other tissues bathed by the oxygen transporting hemoglobin system. The utilization of small amounts of oxygen by the lens[8] does not necessarily reflect the total metabolic activity, since in some tissues such as the lens glycolytic processes not requiring oxygen many furnish a large fraction of the necessary energy requirements. Measurements of the respiratory quotient of the lens (Rq of 1.0)[9, 10] indicate that carbohydrates are the sole substrate for the metabolic requirements.

The major route of glucose breakdown in the lens has been shown to be via the anaerobic cycle or Embden-Meyerhof scheme with the resultant formation of lactic acid as the chief end product of metabolism. This has been demonstrated in several ways. The lactic acid concentrations of the lens and aqueous are considerably higher than plasma. In an eye from which the lens has been removed the level of lactic acid in the aqueous humor is considerably lower than that of the normal eye. Other more direct methods have also shown that Embden-Meyerhof pathway does indeed account for the majority of the glucose utilized by the lens[11, 12, 13, 14, 15].

The hexosemonophosphate shunt has also been shown to play a significant part in the breakdown of glucose in the lens. It has

been estimated that about ten per cent of glucose is metabolized by the shunt and about ninety per cent by the anaerobic cycle[16].

The enzymes of the citric acid cycle have also been shown to be present in the lens[17, 18] but do not seem to play a major role in lens metabolism. However, recent work[19] indicates a much higher concentration of certain lipid substances in the lens than was previously suspected. This may indicate a more important role of the citric acid cycle than has been considered in earlier writings.

The obvious prerequisite of the transport of glucose or other metabolites into the lens before any of the previously mentioned pathways of metabolism may occur need hardly be mentioned. The movement of glucose across a cell surface or a more complex barrier such as is presented by the combination of the lens capsule and the individual interfaces packaged beneath it may either be by a process of passive diffusion along a concentration gradient or it may be controlled in whole or in part by some active process. The lens capsule itself when isolated from the cellular components of the lens has been shown to be selective only in respect to the size of the particles presented to it[20].

Early workers felt that the movement of glucose into the lens was primarily a matter of simple diffusion. It was known that the level of glucose in the lens itself was somewhat below that of the surrounding fluid whether it be the aqueous humor in the in vivo specimen or the artificial medium in the in vitro preparation. Now it is felt that glucose transport into the lens is at least in part metabolically mediated and is not dependent solely on simple diffusion. This conclusion is based largely on the following

observations. First, when bathed in solutions containing a high glucose concentration and such poisons as iodoacetate, fluoride or dinitrophenol the accumulation of glucose within the lens is reduced suggesting that these substances inhibit some active mechanism responsible for glucose transport(21, 22). Second, when the integrity of the lens surface is disrupted as by decapsulation glycolytic activity is reduced(23). Third, glucose moves across the lens capsule more rapidly than another metabolite (acetate) of smaller size(24).

The opposite effect, i. e., an enhancement of glucose movement or utilization has been seen following the addition of insulin to in vitro preparations such as muscle,(25, 26) liver,(27, 28) and adipose tissue(29). Since one of the postulated actions of insulin is to accelerate a step concerned with active transport of glucose into the cell, or to increase cellular permeability to glucose(30, 31, 32) a similar influence on the lens might be anticipated. It had been observed that insulin did not alter the accumulation or uptake of glucose by normal intact lenses(21). The effect of insulin on glucose accumulation in diabetic lenses however, remained in doubt due to the initial high and variable levels of lenticular glucose. The usual procedures for measuring glucose transport are not adapted to the diabetic lens. It seemed likely however that the accumulation of C^{14} from uniformly labeled glucose might provide an answer and our attentions were thus directed to such studies.

Investigations of the effect of decapsulation were incorporated because of the importance of the capsule in any transport mechanism.

As studies along these lines continued and reports from other

laboratories were received, a closely related problem presented itself. Although evidence indicated that insulin had no influence on glucose metabolism of the intact isolated lenses, an in vivo effect on the lens was postulated by other workers (see later). It was felt that this seeming discrepancy could best be resolved by determining whether insulin entered the aqueous humor of the intact animal in physiologically significant concentrations. To this end the accumulation of I^{131} labeled insulin in the aqueous humor was measured employing techniques similarly used to determine the insulin distribution in other tissues(33).

This thesis thus is concerned with several aspects of glucose metabolism of the lens, including the effect of insulin, alloxan diabetes, and decapsulation, and the related question of the presence of insulin in the aqueous humor.

II. MATERIALS AND METHODS

A. Lens Studies:

Rabbit eyes were generally obtained from a local slaughter-house. Immediately after death by decapitation the eyes were enucleated, placed in ice cold saline and transported to the laboratory packed in ice. Lenses were obtained under aseptic conditions by cutting off the optic nerve, turning back the sclera in four equal flaps, removing the vitreous with a lens loop, (care being taken to avoid touching the lens) and carefully incising the zonules with small scissors.

When measuring the accumulation of labeled carbon from uniformly labeled glucose the following procedures were then utilized. The lenses were placed posterior surface down in a 50 ml. flask containing 15 ml. of balanced salt solution similar in ionic composition to aqueous (table 1) and with a glucose concentration of 90 or 200 milligram per cent. One microcurie of uniformly labeled glucose was added to this solution. In certain instances 0.1 unit crystalline insulin per ml. of media was added. The pH of the medium was adjusted to the physiologic range (pH 7.32-7.48).

The incubation container was flushed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, tightly stoppered, and placed in a water bath at 37°C. for a measured period of time.

At the end of the incubation period the lens was removed from the medium, rinsed with medium without added isotope, weighed on a Roller-Smith tissue balance, frozen rapidly and divided equatorially to separate the anterior capsule from the posterior capsule.

In some instances the complete lens substance was macerated evenly over a one inch aluminum planchet. In other cases the anterior lens substance was separated from the posterior lens substance at the time of the equatorial sectioning and each part was spread on a separate planchet. Similarly the anterior and posterior capsules were either placed on separate planchets or placed together. The lens material was dried for one hour at 105°C. and then assayed for radioactivity under a Tracerlab end window counter.

The fraction of the total radioactivity accumulation in the lens which could be attributed to unmodified glucose was determined by paper chromatographic separation.

The lens was incubated in the solution with added isotopic glucose as outlined above. Following incubation the lens was rinsed, weighed, macerated in a test tube, and the proteins precipitated with an ether-alcohol mixture. The supernatant fluid and three water washings of this precipitate were evaporated to dryness over a steam bath and extracted with pyridine at 100°C. for 10 minutes. The supernatant fluid from this procedure was evaporated to dryness under vacuum. This fraction was dissolved in water and applied to Whatman No. 1 filter paper for paper chromatography studies. Unlabeled glucose was added to the origin to provide sufficient glucose for a well defined spot. Various solvents were tried. Isopropyl alcohol, pyridine, water and glacial acetic acid in the proportions of 8:8:4:1 proved to provide the best separation and was employed throughout. In this solvent descending chromatography was carried out for a period of 16 hours. The strips were stained with a solution of 0.5 grams benzidine, 80 ml. absolute alcohol and 200 ml.

glacial acetic acid to outline the glucose spot. (Here again several stains were tried. This proved to be the most efficient.) The entire strip to the solvent front was divided into two by two centimeter sections which were individually assayed for activity under a Geiger-Mueller tube. The non-soluble fractions from the protein precipitation and the pyridine extraction were dissolved in potassium hydroxide, air dried on similar sections of filter paper and counted as were the chromatography strips. The results are expressed as the percentage of the total counts that were present under the area corresponding to the stainable glucose spot.

In studies measuring glucose depletion from the bathing fluid the following procedures were utilized after isolating the lens. When decapsulated lenses were to be studied the capsule was removed from the lens with a pair of small forceps and both the decapsulated lens and its capsule were placed in 3 ml. of the previously listed medium containing 90 milligram per cent glucose. Crystalline insulin (0.1 unit per ml.) was added to certain tubes. The tube was flushed with 95 per cent oxygen and 5 per cent CO_2 gas mixture and incubated at 37°C. After the indicated time the lens was removed from the tube, rinsed, blotted dry and weighed on the Roller-Smith tissue balance. Where the decapsulated lens was employed the weight of this lens was taken to equal that of its intact mate.

For glucose analysis an aliquot of the medium was precipitated with 5 per cent $ZnSO_4$ and 0.3 N $Ba(OH)_2$, diluted to an appropriate concentration and the glucose determined by the method of Somogyi[34].

Glucose depletion* was calculated as the difference between the glucose content of the experimental solution and a control handled in the same manner but without the addition of a lens. It is expressed as milligrams glucose depleted per gram of wet lens per hour of incubation time. Incubation periods were generally six hours unless otherwise noted.

*The term "glucose depletion from the medium" and "glucose uptake by the lens" are used synonymously and here provides a more accurate description than "glucose utilization by the lens". "Glucose accumulation" refers to the amount of unmetabolized glucose accumulating within the lens substance.

B. Aqueous Studies:

To follow the migration of I^{131} labeled insulin into the aqueous after its intravenous administration the procedures listed below were followed. A control sample of blood was obtained by cardiac puncture and I^{131} labeled insulin as received from a commercial source* was injected in the marginal ear vein employing a dosage of 50 microcuries per kilogram body weight. At various time intervals thereafter aqueous and blood were drawn for analysis. Aqueous was withdrawn from the anterior chamber with a 30 gauge needle following the application of a local anesthetic to the exposed surfaces of the eye. Only one sample was taken from each eye except for a few measurements of the activity in the secondary aqueous. Blood was obtained throughout the experiment by cardiac puncture. Preparatory to measuring the activity an equal volume of one per cent serum albumin was added to the aqueous sample and the proteins of both the plasma and aqueous were precipitated with ten per cent trichloroacetic acid. The precipitate was washed three times with five per cent trichloroacetic acid, then dissolved in 30 per cent potassium hydroxide. The wash solution was added to the supernatant and the activity of both the dissolved precipitate and the supernatant solution was measured by means of a shielded Geiger-Mueller tube and scaling unit.

To further identify the activity found in the protein precipitate electrophoretic studies of aqueous and plasma obtained in the same manner were made. Preliminary studies of the migration of the labeled

*Abbott Laboratories

insulin were necessary. Small amounts of this material were subjected to paper electrophoresis using the Beckman Spinco Model R paper electrophoresis system, in barbital buffer, pH 8.6, ionic strength 0.075, at 2.5 ma constant current for 16 hours, using Schleicher and Schnell 2043A-mgl paper, 30 cm. by 30.6 cm. In certain instances carrier insulin or other proteins were added. After electrophoresis the strip was prerinsed with methanol, stained with bromphenol blue in methanol, rinsed three times with five per cent glacial acetic acid and dried. It was then cut into sections one centimeter wide and the activity of each section determined by counting under a shielded Geiger-Mueller tube. In general ten such sections were assayed from each strip. All the activity on the strip was included in this area.

Because of the small amount of protein in the aqueous the usual method of handling specimens for electrophoretic analysis was modified. An aqueous volume of from 0.15 to 0.25 ml. was absorbed on a two mm. wide strip of electrophoresis paper. A six microliter sample of plasma was similarly employed. The strips were air dried and placed at the origin

Generally six microliters of plasma drawn from the animal before the injection of the labeled insulin were applied to the aqueous strip to provide sufficient carrier for non-insulin activity. Electrophoresis, staining, sectioning and counting were performed as previously outlined. Results are presented as counts per minute in 0.2 ml. of aqueous or plasma.

C. General Procedures:

Animals were made diabetic by the intravenous administration of five per cent alloxan solution in a dosage of 175 milligrams per kilogram body weight. On the fifth day the level of the blood sugar was determined. Only those rabbits with a level of glucose above 400 milligram per cent were used. To protect the rabbits from the effect of the hypoglycemia that followed the injection of the alloxan, they were given ten to fifteen ml. of ten per cent glucose per kilogram body weight intraperitoneally at time periods of eight, twelve and sixteen hours following the alloxan administration. A modification that proved successful in later parts of the study was to give the glucose solution at only eight and fourteen hours with a compensatory increase in the dose of glucose. A glucose solution was substituted for the drinking water for the first 24 hours following the alloxan.

In some normal animals the influence of high glucose levels on the migration of insulin was studied. Exogenous hyperglycemia was induced by the combined intravenous and intraperitoneal administration of 50 per cent glucose solution. Blood glucose levels were maintained above 400 milligram per cent during this experimental procedure.

III. RESULTS

A. C^{14} accumulation studies:

As the study of the accumulation of isotopic carbon from uniformly labeled glucose by the lens progressed it became apparent that under identical conditions the weight (age) of the lens significantly influenced the amount of activity recorded. Apart from the variation noted in individual lenses it was found that as lens weight increased the recorded accumulation of activity per lens decreased in a linear fashion (fig. 1). Several factors may account for this observation. First, with increasing lens age there is a corresponding decrease in metabolic activity. Second, as the lens grows volume to surface ratio increases. Third, as the mass of lens substance increases self absorption of the activity is greater and the counting technique less reliable. The decreasing counts recorded when lens weight increases makes it impossible to directly compare one experimental situation with another as absolute counts per minute per lens when the weights of the lenses in the two situations differ to any extent. However, by multiplying counts per minute per lens by lens weight a constant relationship was obtained. This permits one to compare directly lenses of different weights (fig. 1). This relationship was found whether lenses were from diabetic or normal animals.

The spread in counts per minute per lens noted at any one lens weight was in large extent due to variation between different rabbits. Paired lenses (from the same animal) run under identical conditions showed good agreement. Here as in all lens studies the

two lenses from the same animal should ideally make up the complete experiment, i. e., one lens as the control and the other as the test situation. Obviously under certain conditions groups of animals must be compared with each other, as the comparison of normals with diabetics. For this reason the previously mentioned empirical step of multiplying lens weight by counts per minute accumulated per lens was undertaken to allow different groups of animals to be directly compared.

One of the first problems was to determine if the time course of C^{14} accumulation closely enough approximated the time course of the accumulation of fermentable reducing substance previously reported(21) in order that C^{14} accumulation from a medium containing uniformly labeled glucose may be taken as another index of glucose transport across the lens surface. Normal rabbit lenses were incubated in a medium containing uniformly labeled glucose for various time intervals to determine the kinetics of the accumulation of C^{14} by the lens substance (fig. 2). The rate of accumulation of the labeled carbon becomes progressively slower until by 24 hours it is essentially at a steady state. This curve closely follows the one previously obtained when the accumulation of reducing substances by the lens was measured. We have accordingly considered that C^{14} accumulation from a medium containing uniformly labeled glucose is an index of glucose transport into the lens. At six and twelve hour incubation periods the accumulation of activity from a medium containing 200 milligram per cent glucose was the same as that from one containing 90 milligram per cent glucose (the same amount of labeled glucose being employed). Thus the concentration of unlabeled glucose does not influence the uptake of the label. The technique

is thus applicable to the diabetic lens when a high glucose concentration is encountered.

The amount of isotope accumulated in the lens capsule is shown in figure 3. On a per weight basis the activity accumulated by the capsule was considerably greater than that of the lens substance itself. This would be expected from the spatial relationship of the capsule to the medium as compared to the remainder of the lens. A higher metabolic activity near the surface may also be a factor. When the anterior and posterior capsules were counted separately generally 50 to 100 per cent more activity was found in the anterior capsule. In a small series the lenses were divided equatorially and the posterior and anterior segments were counted individually. A significantly greater activity was found in the anterior portion.

To estimate the percentage of the accumulated activity in the lens contributed by labeled but unmetabolized glucose paper chromatographic separation was undertaken. That activity of the lens which migrated a distance corresponding to a stainable glucose spot was measured and compared to the total activity. It was found that about sixty-two per cent of the activity migrated to a point corresponding to the glucose spot on the paper strip (table 2).

Paired lens systems were used to measure the effect of insulin on the accumulation of C^{14} by both normal and diabetic intact lenses. The lenses were incubated for six hours at 37°C. in a medium containing 200 milligram per cent glucose. Insulin was added to one lens of the pair in the concentration of 0.1 unit of crystalline insulin per milliliter of incubation fluid. No significant effect of insulin was noted on the uptake of activity by either the normal (fig. 4)

or diabetic (fig. 5) lenses. Using the function of lens weight times counts accumulated the diabetic lenses showed a slight but significantly higher level of activity than did the normal lenses (fig. 6).

B. Glucose depletion studies:

The normal intact lens has an uptake of glucose of slightly over 0.7 milligram per gram of wet lens per hour of incubation time when incubated for six hours. Upon removal of the capsule this uptake was reduced to about 55 per cent of the normal value (fig. 7).

The uptake of glucose by intact diabetic lenses was considerably lower than that of the normal lens. Decapsulation of the diabetic lens further reduced the amount of glucose removed from the medium. In fact in several decapsulated lens systems the glucose level of the fluid was slightly higher following the incubation period than it was prior to incubation. It seems probable that these findings are in large part due to the initial high level of glucose in the diabetic as compared to the normal lens. In one series of five rabbits where blood sugar levels ranged from 360 milligram per cent to greater than 960 milligram per cent at the time of sacrifice an excellent correlation was noted between the decreasing uptake of glucose by both intact and decapsulated lenses as the blood sugar level of the animals increased.

As had been previously noted[21] the addition of insulin to the intact lens system caused no increase in glucose uptake. However, the addition of 0.1 unit of crystalline insulin per milliliter of incubation fluid to a decapsulated lens increased glucose depletion by about 28 per cent over its paired lens without insulin.

C. Labeled insulin studies:

After intravenous injection of fifty microcuries of I^{131} labeled insulin per kilogram body weight into rabbits the level of both trichloroacetic acid precipitable and soluble activity was measured. The precipitable activity in the plasma decreased rapidly after the injection of the labeled insulin (fig. 8). The soluble activity in plasma rose to a maximum about one hour following the administration of the insulin and then slowly decreased.

The precipitable activity in the aqueous rose to a peak at about 45 minutes and then gradually dropped to low levels (fig. 9). A few measurements of the precipitable activity in the secondary aqueous showed considerably higher levels of activity as would be expected.

Although nearly 100 per cent of the biologically active insulin is precipitated by trichloroacetic acid,(35) the converse, namely that all activity in the coaglum represents insulin, cannot be assumed.

Impurities present in the insulin or other substances to which released I^{131} may become bound may also be found in the precipitate. To determine the true insulin a further step to distinguish between I^{131} attached to insulin and that attached to other trichloroacetic acid precipitable material is essential. For this purpose electrophoretic separation of the protein constituents on paper was employed.

To determine the feasibility of identifying I^{131} labeled insulin by means of paper electrophoresis preliminary studies of the electrophoretic behavior of the anticipated amounts of labeled insulin under certain conditions were necessary. Small amounts of labeled insulin were subjected to paper electrophoresis. In certain instances

carrier unlabeled insulin or other proteins were added. After electrophoresis the strip was assayed for activity.

Labeled insulin alone remained near the origin following electrophoresis (fig. 10). When carrier insulin was added the activity moved down the strip to a point corresponding to that of the stainable band of insulin. However, albumin and insulin migrated at approximately the same rate. Hence separation of activity due to insulin and albumin is not feasible by this technique.

With added serum or plasma the activity remained at the origin and the next adjacent strip. It appears therefore that the most exact determination of the activity due to insulin was achieved by analyzing unaltered plasma samples or aqueous samples to which plasma was added as a carrier for any non-insulin activity. Under these circumstances the activity remaining within the first two sections was considered to be insulin activity. Additional weight is given to the assumption that the activity remaining at the origin is insulin by the findings of Scott et al(36) who showed that the biologic activity of plasma insulin paralleled the level of I^{131} activity remaining at the origin under similar conditions.

Radioelectrophoretograms of plasma samples drawn at progressive time intervals following intravenous administration of labeled insulin are shown in figure 11. No significant difference was seen in the amount of activity migrating with the plasma proteins at the various time intervals. However the total activity presumed to be insulin showed a progressive decline with time.

Relatively similar pattern were seen in radioelectrophoretograms of aqueous taken at progressive time intervals (fig. 12). A small but significant amount of activity remained at the origin. The probability that this material was insulin was confirmed by adding carrier insulin. When this was done the activity migrated down the strip (fig. 13) whereas no movement was noted without the carrier. As with plasma samples there was no significant difference with time in the amount of activity moving down the strip with the carrier plasma protein.

The rate of insulin degradation in the plasma and its entry into the aqueous was estimated by plotting the activity remaining in the first two sections of the strips against time (fig. 14). As with the trichloroacetic acid precipitable activity a rapid decrease in insulin activity in the plasma was noted. In the aqueous a rise in insulin activity to a peak level at 30-60 minutes was observed followed by a gradual decline at later time periods. It should be noted that the activity in the aqueous humor attained only a small fraction (about 1/100th) of that found in the plasma.

No significant difference from the normal was seen in the time course of plasma or aqueous insulin activity in diabetic (fig. 15) or exogenously hyperglycemic animals (fig. 16). When a composite was made of the three situations together with values from a normal rabbit pretreated with insulin (0.5 unit per kilogram) prior to injection of the labeled insulin the plasma values of insulin activity closely overlapped (fig. 17).

A similar composite of aqueous insulin activity showed a wider spread of the points but no outstanding difference between the groups (fig. 18).

The radioelectrophoretic patterns of plasma samples from these various situations showed essentially the same migratory patterns (fig. 19).

IV. DISCUSSION

If the lens were like many other tissues it might be expected that insulin addition to the in vitro system would result in an increased movement of glucose into the lens. However, no effect of insulin on either the accumulation of fermentable reducing substances or C^{14} from uniformly labeled glucose by the intact lens has been noted. In addition diabetic preparations which in other tissues generally show a good response to insulin showed no increased accumulation of the isotope by in vitro insulin addition.

These observations of the lack of an in vitro insulin effect on the lens have recently been strengthened by studies utilizing intact normal and diabetic rat lenses. Farkas and Patterson(37) reported a lack of insulin effect on glucose depletion by these isolated lenses.

The lens thus resembles the erythrocyte into which glucose moves at a more rapid rate than would be predicted from its molecular size. Insulin does not influence this movement(38).

This lack of an insulin effect in the in vitro intact lens may be due to one or more factors. First, insulin may not enter the aqueous humor in physiologically significant quantities and the potential for its use by the lens therefor has not been developed. Secondly, insulin as presented to the lens in the artificial in vitro preparation may not be the active principal normally needed by the lens, i. e., alteration of its structure in some way may be necessary before it becomes active. Third, a cofactor not present in the lens itself but normally supplied by other tissues may be necessary before insulin acts on lenticular metabolism.

Fourth, insulin although present in the aqueous humor and the bathing medium is prevented from acting on the lens because an impermeable capsule does not permit penetration of the hormone.

Farkas and Patterson further reported that when rats were pretreated with insulin prior to isolation of the lens an increased glucose uptake was observed by normal or diabetic lenses. (It has also been shown that pretreatment of rats with insulin results in a marked increase of glycogen formation by liver slices, whereas when insulin was added to the medium no effect was noted(39).) To further compound the question of insulin effect on the lens they found that this facilitory effect of pretreatment could nearly be abolished by removal of the liver. This observation opens up at least three possible mechanisms of action. First, the liver may produce a cofactor that in conjunction with the insulin molecule causes an increased glucose uptake. Second, the liver may alter the insulin molecule to some active form that acts upon the lens. Third, the stimulation of the administered insulin may result in the formation of an unknown substance that acts on the lens.

The measurement of the insulin levels in the aqueous humor gives at least a partial answer to the question of which of these mechanisms may be operable. The level of activity which can be considered biologically active insulin (as we know it for other tissues) that enters the aqueous has been shown to be considerably lower than the plasma levels following the intravenous injection of labeled insulin. It is considered that insulin does not enter the aqueous in physiologically significant quantities and the tissues bathed by this fluid are in a state of relative diabetes at all times (using

the insulin levels normally available to other tissues as a baseline). However the possibility that in some way much lower levels of insulin will suffice for normal functions in these tissues must not be ignored.

The findings that a substance identifiable as insulin by paper electrophoresis was not present in significant quantities in the aqueous seems to rule out the possibility that the effect of the liver is to supply a cofactor to the insulin which in combination acts on the lens. If the liver modifies the insulin molecule in some fashion it must do so to such an extent that it no longer has the electrophoretic characteristics of the parent structure. This seems unlikely. The suggestion that pretreatment stimulates the production of an active factor by the liver is a question not yet answered. Whether this is a species specific effect must be considered.

The disruption of lenticular integrity by decapsulation results in marked changes in the metabolic pattern of the lens. Although oxygen consumption by the lens increases with decapsulation(40) other major functions decrease following this maneuver. Glucose uptake decreases to about 55 per cent of its normal level. Decreased activity is also evidenced by a decreased production of lactic acid,(41) and a decreased accumulation of labeled phosphorus(42) by the lens substance of a decapsulated lens.

The presence of the lens capsule is thus essential for the maintenance of proper cell life in vitro. In vivo rupture of the capsule results in a progressive absorption of the lens substance by the aqueous humor--a process undoubtedly similar in part seen to the progressive disintegration of the decapsulated lens in vitro.

This lessening of activity when the capsule is removed must in large part be due to a destruction of cellular integrity.

An earlier report[43] indicated that the presence of insulin in the incubation fluid increased glucose depletion by the decapsulated lens to 350 per cent of its control level. Another report[21] of insulin action on a disrupted lens system showed a smaller but significant effect of insulin on glucose depletion in lenses with capsular integrity destroyed by an incision through the capsule. The findings of about a 28 per cent increase of glucose depletion in decapsulated lenses with added insulin more closely corresponds to the degree of increase found in the nicked capsule study than the former study of decapsulated lenses.

Why this action of insulin is now seen in glucose depletion by the lens when cellular integrity is destroyed is not known. Possibly the capsule is an absolute barrier to the action of insulin and prevents it from moving to the cells beneath. With removal of this barrier insulin becomes effective. Rupture of the lens fibers either during decapsulation or during the incubation period may allow insulin to act on intracellular units now released to the fluid. Williams et al has shown that insulin distributes itself in a characteristic intracellular pattern following intravenous injection into rats[44, 45, 46]. However this again is an in vivo situation with intact cells rather than the situation in a decapsulated lens.

Many examples of the depressant effect of diabetes on glucose utilization by various tissues may be found in the literature[47, 48, 49]. In the lens from the diabetic animal the glucose depletion is markedly reduced. However as mentioned in RESULTS this reduction

may in large part be due to the high level of glucose in the lens itself at the start of the incubation period. The simple explanation for the reduced glucose depletion by the diabetic lens does not explain why the isotopic carbon accumulation by these tissues is not depressed.

Other points mentioned briefly in RESULTS should be amplified at this time.

The higher level of activity found in the anterior capsule is most likely due to the attachment of the highly active epithelial cells to this section of the capsule and the accumulation of the activity by these cells. The presence of a higher level of activity in the anterior portion of the lens substance is not surprising in the light of the knowledge of the more active surface of the lens being anteriorly. Similar studies measuring the accumulation of P^{32} in different sections of the lens have also indicated that the activity in the anterior segment accumulates more rapidly(50). As mentioned earlier, teleologic reasoning must give the anterior surface the more active role because of the rapid turnover of nutrients across its surface as compared to the relatively stable posterior surface.

Of particular interest is the fact that both the rate of degradation of insulin activity in the plasma and the rise and fall of activity in the aqueous is similar in all the situations tested whether from normal, diabetic, hyperglycemic or insulin pretreated animals. Similar results have been reported in studies on humans, which demonstrated that normals, untreated diabetics and patients with other diseases all showed similar rates of insulin degradation(51).

It is interesting to note that insulin enters the cerebrospinal fluid in insignificant amounts(52) as well as the aqueous and probably is not required for function of the central nervous system. Investigations have shown that the blood-aqueous barrier is essentially similar to the blood-cerebrospinal fluid barrier(53), and in the light of this it is not surprising that insignificant quantities of insulin would be present in both fluids. One can further speculate that insulin does not cross the blood-retinal barrier either, since this is similar physiologically to the blood-brain barrier(54). That the retinopathy of diabetes seems unrelated to the insulin requirement, therefore, is not surprising.

SUMMARY

1. Recorded C^{14} accumulation by lenses decreased as lens weight increased.

2. Insulin had no effect on C^{14} accumulation from uniformly labeled glucose by either normal or diabetic lenses.

3. The anterior portion of both the lens capsule and lens substance accumulated more activity than did the corresponding posterior portions.

4. Approximately 62 per cent of the accumulated activity migrated to a point corresponding to a stainable glucose spot on paper chromatography.

5. Decapsulation resulted in a lowered glucose uptake by both normal and diabetic lenses. Diabetic lenses (intact or decapsulated) depleted less glucose from the medium than did normal lenses.

6. Insulin addition to a decapsulated lens increased its glucose uptake.

7. Only small amounts of insulin enter the aqueous humor after the intravenous injection of labeled insulin.

8. No significant difference was noted in the rate of plasma degradation of the injected insulin in diabetic or normal animals.

9. The significance of these findings are discussed.

BIBLIOGRAPHY

1. Giles, N.M., Harris, J.E. Radioelectrophoretic patterns of aqueous and plasma following intravenous administration of I^{131} labeled insulin. Am. J. Ophth., 46: 196-204, 1958

2. Wood, C.A. The Tadjkirat of Ali ibn Isa of Baghdad, Memorandum Book of a Tenth Century Oculist. Northwestern University, Chicago, 1936

3. Piris, A. Composition of ox lens capsule. Biochem. J., 48: 368-371, 1951

4. Harris, J.E., and Gohrsitz, L.B. The significance of changes in potassium and sodium content of the lens: A mechanism of lenticular intumescence. Am. J. Ophth., 34: 131-138, 1951

5. Nordmann, J., Mandel, P., and Achard, M. Inhibition of sugar metabolism in the lens. Brit. J. Ophth., 38: 673-679, 1954

6. Patterson, J.W. The effect of high carbohydrate levels on the lens. ACTA XVII, Cong. Ophth., 2: 992-994, 1954

7. Patterson, J.W. Effect of blood supply on cataracts. Am. J. Physiol., 180: 495-497, 1955

8. Field, J. Jr., Tainter, E.G., Martin, A.W., and Belding, H.S. Studies on the oxygen consumption of the rabbit lens and the effect of 2-4 dinitrophenol thereon. Am. J. Ophth., 20: 779-784, 1937

9. Fischer, F.P. Ein versuch den energiewechsel des auges zu bestimmen. Ber. u.d. Versamml. d. deutsch. ophth. Gesellsch. 48: 95-99, 1930

10. Frohman, C.E., and Kinsey, V.E. Studies on the crystalline lens. AMA Arch. Ophth., 48: 12-18, 1948

11. Mandel, P., and Israelewics, D. Studies on glycolysis in the crystalline lens. Determination of phosphofructokinase, hexokinase, aldolase, and triosphosphate dehydrogenase in crystalline lens of calves. Comp. Rend. Acad. Sc., 238: 404-406, 1954

12. Kinoshita, J.H. Carbohydrate metabolism of lens. AMA Arch. Ophth., 54: 360-368, 1955

13. Green, H., Becker, C.A., and Leopold, I.H. Anaerobic carbohydrate metabolism of the crystalline lens. I. Glucose and glucose-6-phosphate. Am. J. Ophth., 39: 106-113, 1955

14. Green, H., Becker, C.A., and Leopold, I.H. Anaerobic carbohydrate metabolism of the crystalline lens. II. Fructose diphosphate. Am. J. Ophth., 39: 113-117, 1955

15. Green, H., Becker, C.A., and Leopold, I.H. Anaerobic carbohydrate metabolism of the crystalline lens. III. Triose phosphate, phosphoglycerate, and phospho-enol pyruvate. Am. J. Ophth., 40: 237-243, 1956

16. Kinoshita, J.H., and Wachtl, C. A study of the C^{14} glucose metabolism of the rabbit lens. J. Biol. Chem., 223: 5-7, 1956

17. Ely, D.C. The oxidation of lactic acid, pyruvic acid, and various members of the citric-acid cycle by bovine lens homogenates. Am. J. Ophth., 34: 127-130, 1951

18. Karkas, I., and Potts, A. On the presence of condensing enzyme in mammalian lens. Am. J. Ophth., 41: 689-690, 1956

19. van Heyningen, R., and Pirie, A. Acid-soluble phosphate in the lens. Biochem. J., 68: 18-28, 1958

20. Fridenwald, J.S. Permeability of lens capsule. Arch. Ophth., 3: 182-193, 1930

21. Harris, J.E., Hauschildt, J.D., and Nordquist, L.T. Transport of glucose across the lens surface. Am. J. Ophth., 39: 161-169, 1955

22. Muller, H.K. Uber die resorption von glucose und askorbinsaure in die linse. Arch. f. Ophth., 140: 258-268, 1939

23. Michael, D., and Vanoce, P. Nouvelles resherches sur le pouvoir glycolytique du cristallin. Compt. Rend. Soc. de Biol. 109: 1011-1012, 1932

24. Heinrichs, D.J., and Harris, J.E. Lens metabolism as studied with the reversible cation shift. IV. The ability of various metabolites to replace glucose. Am. J. Ophth., 42: 358-362, 1956

25. Haft, D.E., and Mirsky, I.A. The action of barbiturate on the carbohydrate metabolism of the isolated rat diaphragm. J. Pharmacol., 105: 74-79, 1952

26. Randle, P.J. The assay of insulin in vitro by means of the glucose uptake of the isolated rat diaphragm. J. Endocrin., 14: 82-86, 1956

27. Haft, D.E., Miller, L.L. Demonstration of direct effects of insulin on the isolated perfused diabetic liver. Biochemica et Biophysica ACTA 19: 386-390, 1956

28. Villee, C.A., and Hastings, A.B. The metabolism of C^{14} labelled glucose by the rat diaphragm in vitro. J. Biol. Chem. 179: 673-687, 1949

29. Krahl, M.E. The effect of insulin and pituitary hormones on glucose uptake in muscle. An. New York Acad. Sc., 54: 649-667, 1957

30. Levine, R., Goldstein, M.S., Huddlestun, B., and Klein, S.P. Action of insulin on the "permeability" of cells to free hexoses, as studied by its effect on the distribution of galactose. Am. J. Physiol. 163: 70-76, 1950

31. Stadie, W.C. Current concepts of the action of insulin. Physiol. Rev. 34: 52- , 1954

32. Wick, A.N., and Drury, D.R. Action of insulin on the permeability of cells to sorbitol. Am. J. Physiol. 166: 421-423, 1951

33. Welch, C.W., Henley, E.D., Williams, N.H., and Elgee, N.J. The distribution and metabolism of insulin labeled with radioactive iodine (I^{131}) in normal and diabetic subjects. Diabetes, 5(1): 15-18, 1956

34. Somogyi, M. A new reagent for the determination of sugars. J. Biol. Chem., 160: 61-68, 1945

35. Berson, S.A., Yalow, R.S., Bauman, A., Rothschild, M.S., and Newerly, K. Insulin I^{131} metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. 35: 170-190, 1956

36. Scott, G.W., Prout, T.E., Weaver, J.A., and Asper, S.P. A comparison of the behavior of insulin and insulin labelled I^{131} in serum. Diabetes, 7: 38, 1958

37. Farkas, T.J., and Patterson, J.W. Insulin and the lens. Am. J. Ophth., 44: 341-346, 1957

38. LaFevre, P.G., and LaFevre, M.E. The mechanism of glucose transfer into and out of the red cell. J. Gen. Physiol., 35: 891-906, 1952

39. Nakai, H. Influence of oxomalate and various hormones on the carbohydrate metabolism in rat liver slices (in vitro). Endocrinol. Japon., 3:23-26, 1956

40. Ely, L.O. Metabolism of the crystalline lens. II. Respiration of the intact lens and its separated parts. Am. J. Ophth., 32: 215-219, 1949

41. Muller, H.K., and Kleifeld, O. Berichte d. Deutch Ophth. Gesa. 58: 214, 1953

42. Pirie, A., and van heyningen, R. Biochemistry of the Eye. Charles C. Thomas, Springfield, Illinois, 1956

43. Ross, E.J. Insulin and the permeability of cell membranes to glucose. Nature 171: 125-126, 1953

44. Elgee, N.J., Williams, R.H., Lee, N.D., Wong, T., and Hegness R. Studies of radioactive insulin. Diabetes, 2: 370-371, 1953

45. Lee, N.D., and Williams, R.H. The intracellular localization of labelled thyroxine and labelled insulin in mammalian liver. Endocrin. 54: 5-19, 1954

46. Elgee, N.J., Williams, R.H., and Lee, N.D. Distribution and degradation studies with insulin I^{131}. J. Clin. Invest., 33: 1258-1260, 1954

47. Teng, C.T. Studies on the carbohydrate metabolism in rat kidney slices. II. Effect of alloxan diabetes and insulin administration on glucose uptake and glucose formation. Arch. Biochem., & Biophysics, 48: 415-420, 1954

48. Goranson, E.S. and Krulkar, S.D. Effect of insulin on the aerobic phosphorylation of creatine in tissues of alloxan diabetic rats. Arch. Biochem., 24: 40-48, 1949

49. Renold, A.E., Hastings, A.B., and Nesbett, F.B. Studies on carbohydrate metabolism in rat liver slices. III. Utilization of glucose and fructose by liver from normal and diabetic animals. J. Biol. Chem., 209: 687, 1954

50. Palm, E. On the phosphate exchange between the blood and the eye. ACTA Ophth. Suppl. 32, 1948

51. Welsh, C., Henley, E., Williams, R.H., and Cox, R.W. Insulin I^{131} metabolism in man. Am. J. Med., 21: 334-338, 1956

52. Haugaard, N., Vaughan, M., Haugaard, E.S., and Stadie, W.C. Studies of radioactive injected labelled insulin. J. Biol. Chem., 208: 549-563, 1954

53. Davson, H. A comparative study of the aqueous humor and cerebrospinal fluid in the rabbit. J. Physiol., 129: 111-122, 1955

54. Palm, E. On the occurance in the retina of conditions corresponding to the blood-brain barrier. Acta Ophth., 25: 39-42, 1947

Figure 1. Effect of lens weight on C^{14} accumulation after incubation at 37°C. for six hours in a medium containing 200 milligram per cent unlabeled glucose and one microcurie uniformly labeled glucose.

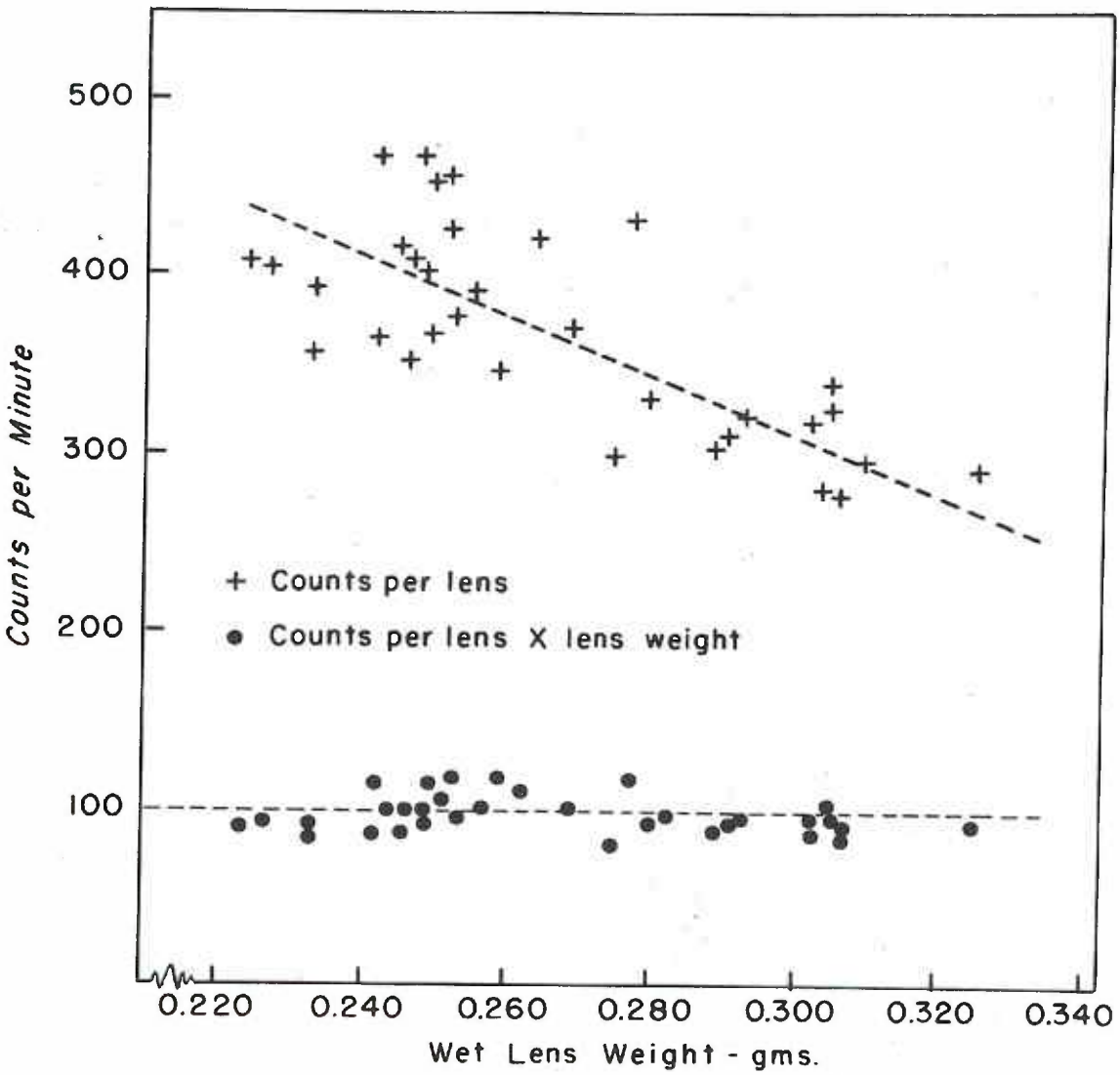
500
400
300
200
100
Counts per Minute
+ Counts per lens
• Counts per lens X lens weight
0.220
0.240
0.260
0.280
0.300
0.320
0.340
Wet Lens Weight - gms.

Figure 2. Time course of C^{14} accumulation by lenses incubated at 37°C. in a medium containing 200 milligram per cent unlabeled glucose and one microcurie uniformly labeled glucose.

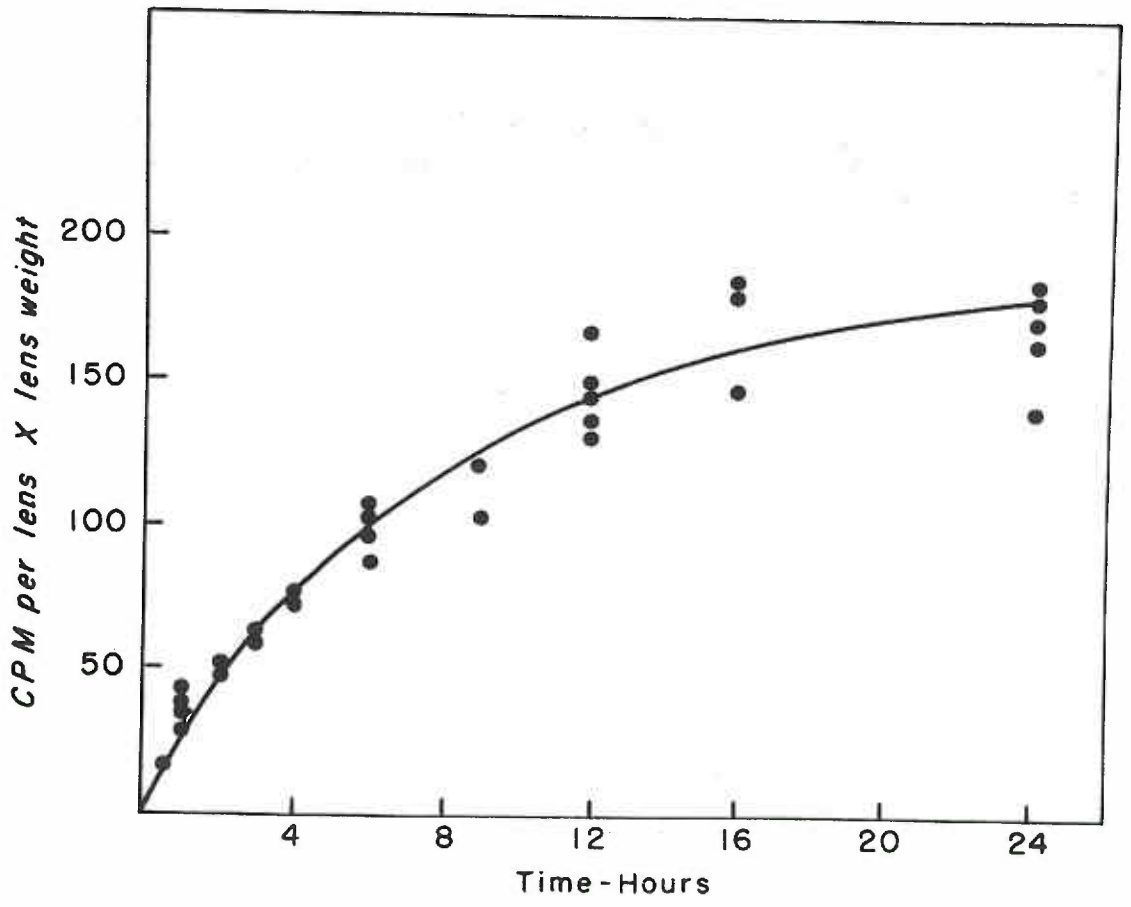
CPM per lens X lens weight
200
150
100
50
4
8
12
16
20
24
Time - Hours

Figure 3. Time course of C^{14} accumulation by the lens capsule incubated at 37°C. in a medium containing 200 milligram per cent unlabeled glucose and one microcurie uniformly labeled glucose.

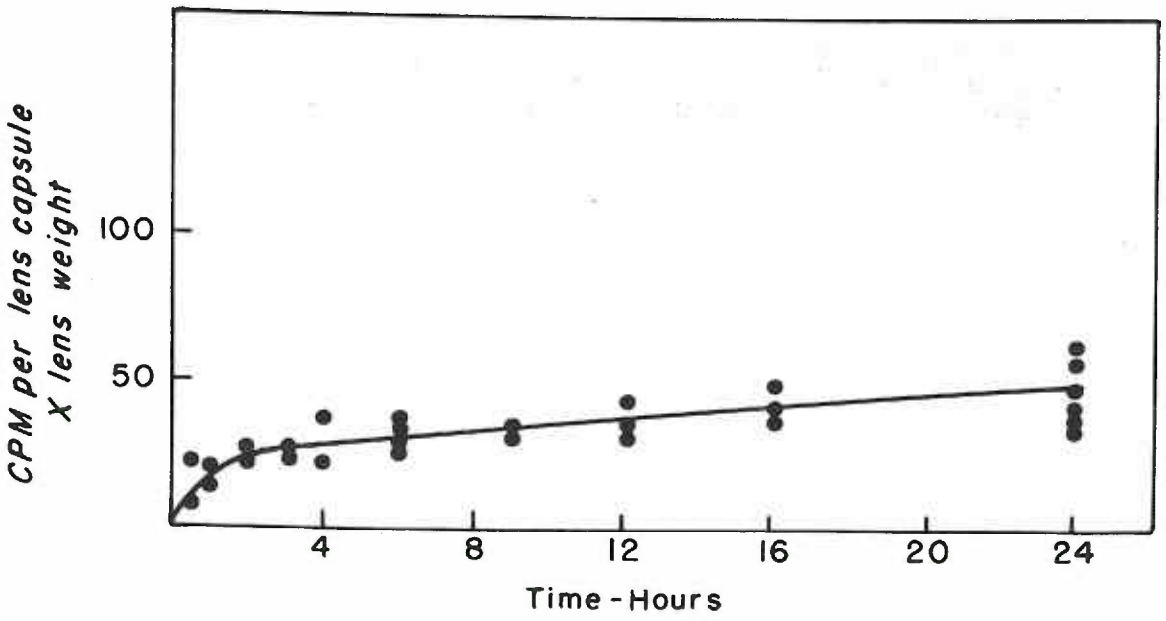
CPM per lens capsule
X lens weight
100
50
4
8
12
16
20
24
Time - Hours

Figure 4. Effect of insulin on the accumulation of C^{14} from uniformly labeled glucose by normal lenses incubated at 37°C. for six hours in a medium containing 200 milligram per cent unlabeled glucose and one microcurie of uniformly labeled glucose.

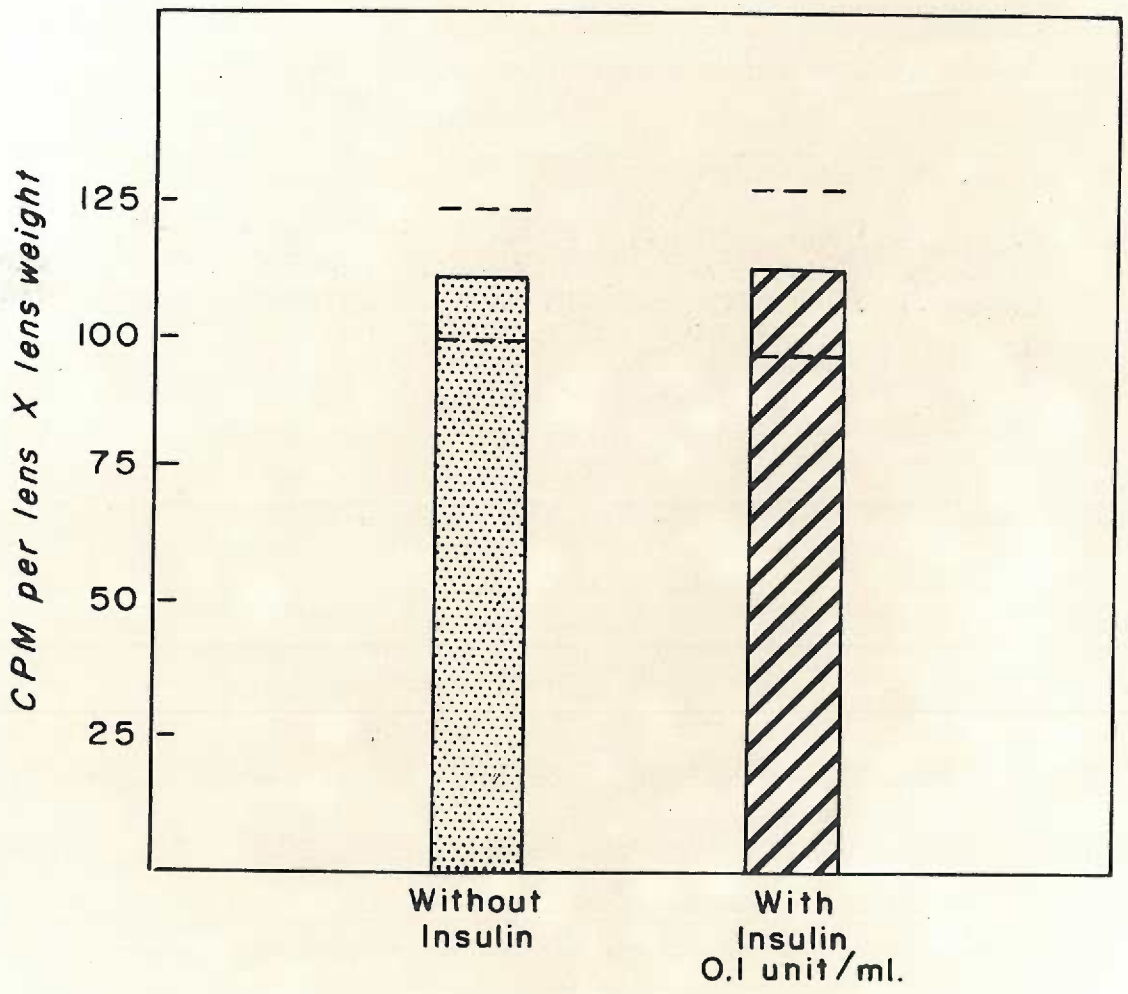
CPM per lens X lens weight
125
100
75
50
25
Without Insulin
With Insulin 0.1 unit/ml.

Figure 5. Effect of insulin on the accumulation of C^{14} from uniformly labeled glucose by diabetic lenses incubated at 37°C. for six hours in a medium containing 200 milligram per cent unlabeled glucose and one microcurie of uniformly labeled glucose.

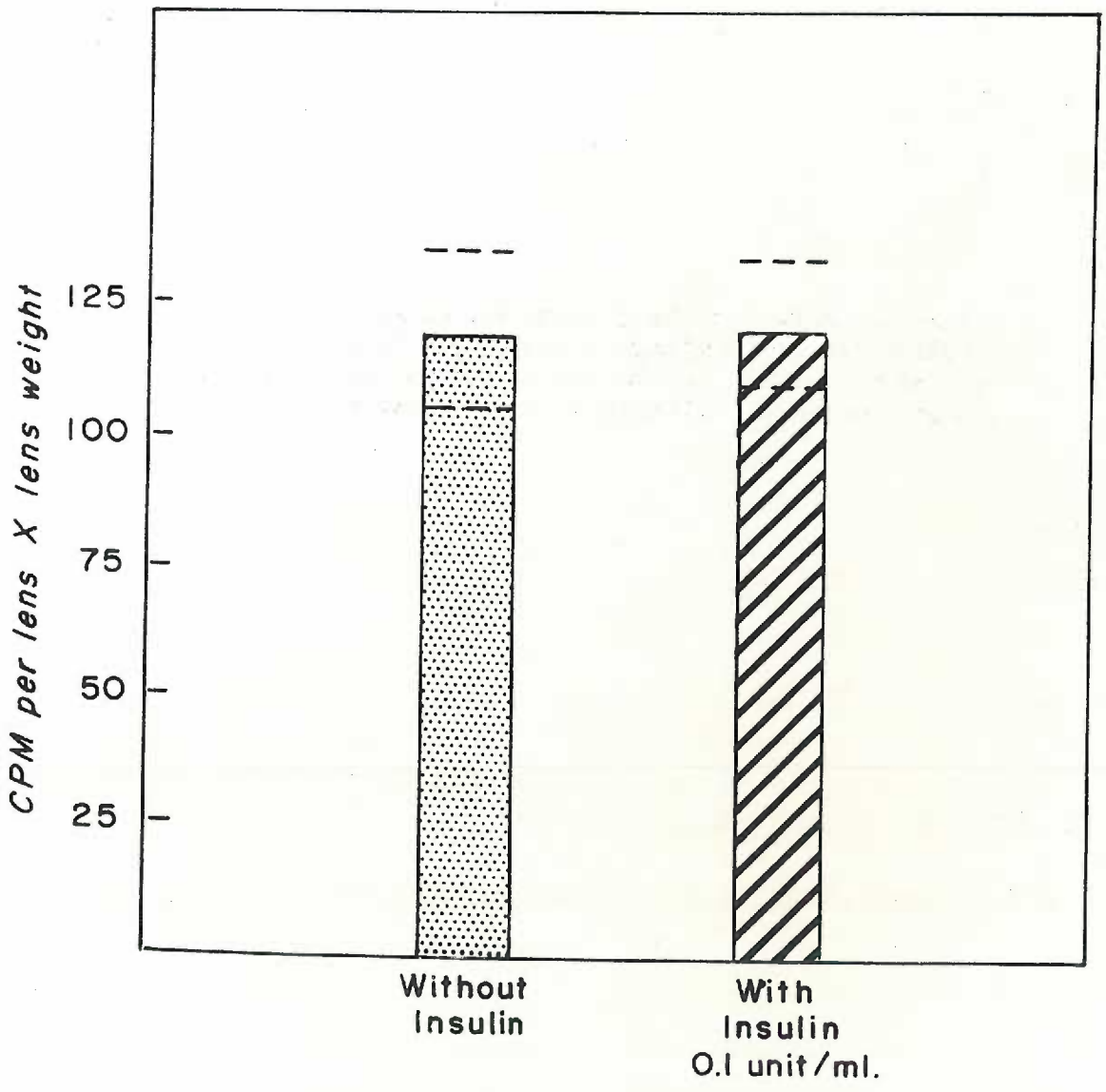
CPM per lens X lens weight
125
100
75
50
25
Without Insulin
With Insulin 0.1 unit/ml.

Figure 6. Comparison of the C^{14} accumulation of diabetic and normal lenses incubated at 37°C. for six hours in a medium containing 200 milligram per cent of unlabeled glucose and one microcurie of uniformly labeled glucose.

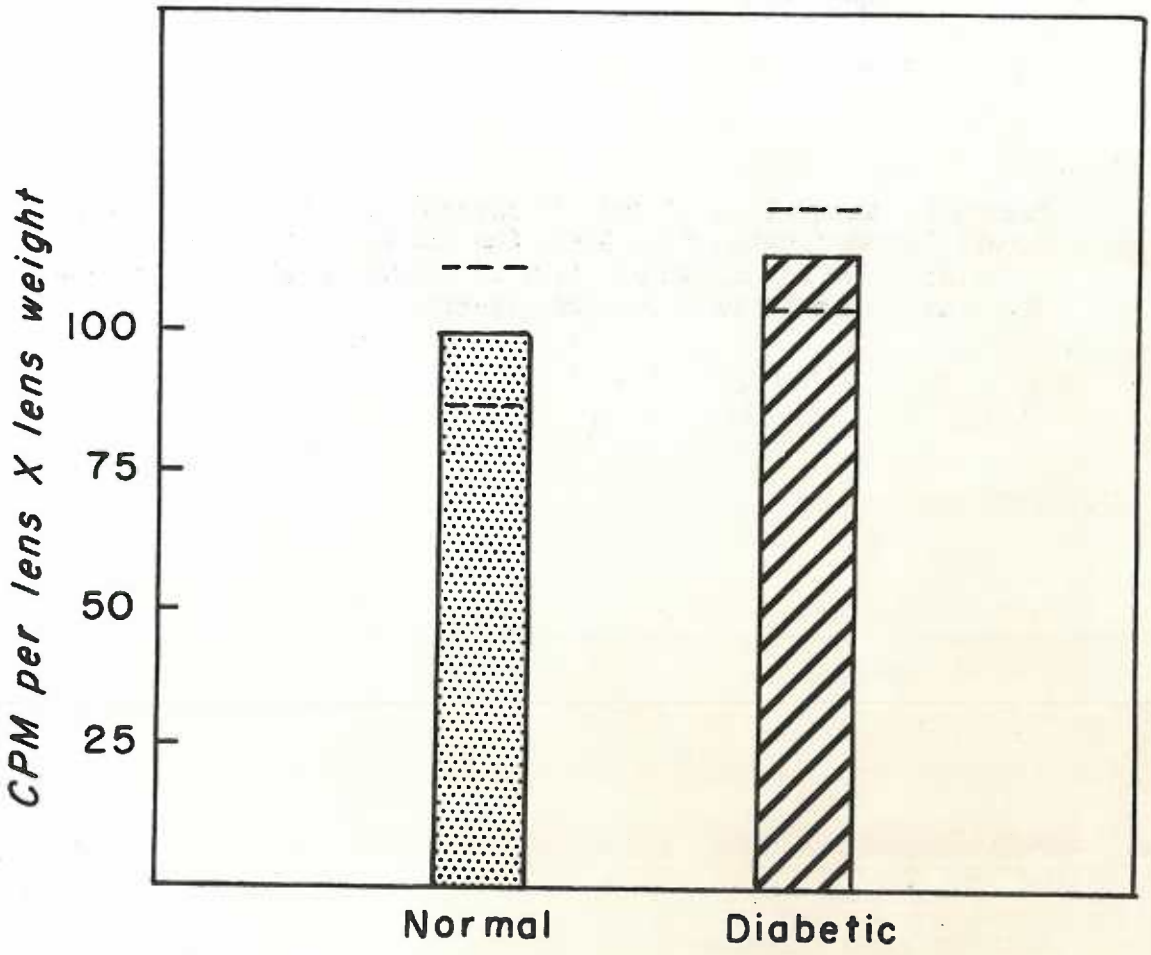
CPM per lens X lens weight
100
75
50
25
Normal
Diabetic

Figure 7. Effect of decapsulation on glucose depletion by lenses incubated for six hours at 37° C. in a medium containing 90 milligram per cent glucose.

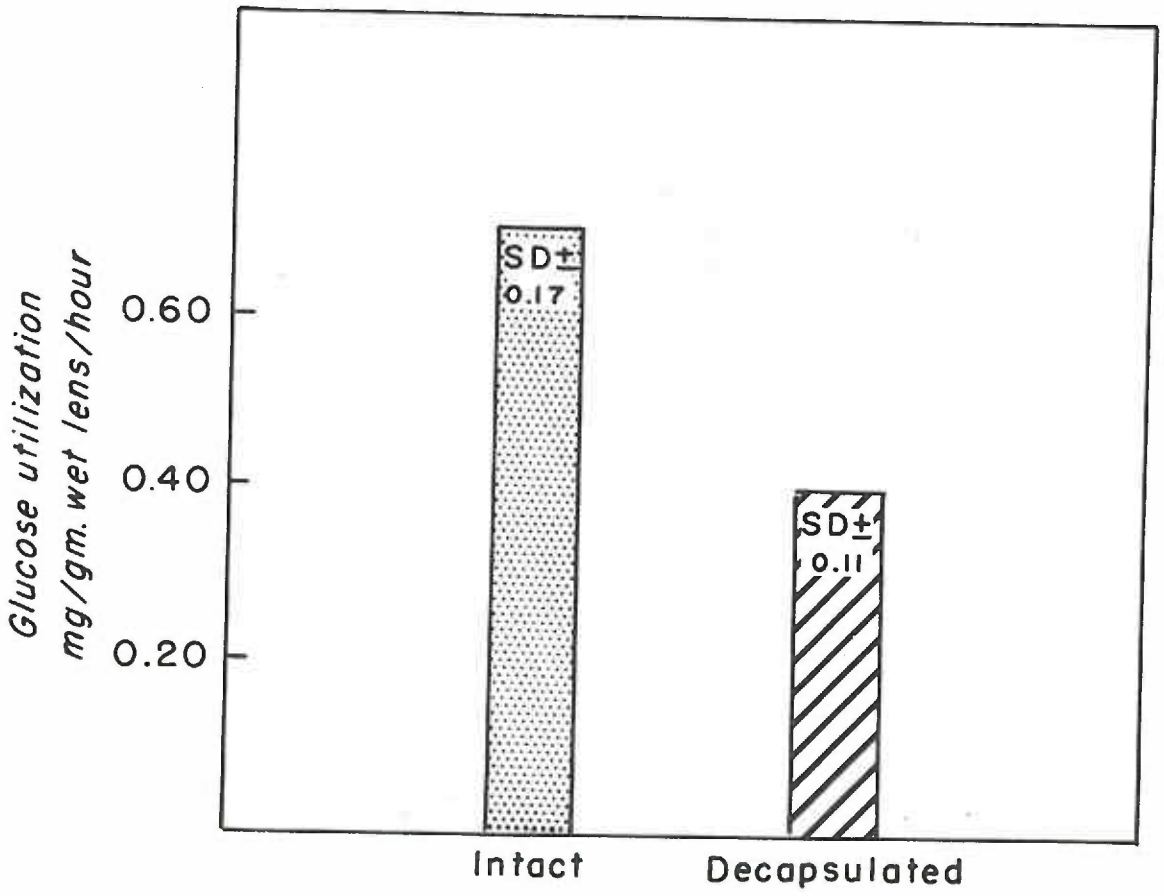
Glucose utilization
mg/gm. wet lens/hour
0.60
0.40
0.20
SD±
0.17
SD±
0.11
Intact
Decapsulated

Figure 8. Time course of trichloroacetic acid precipitable and soluble activity in plasma following intravenous injection of I^{131} labeled insulin.

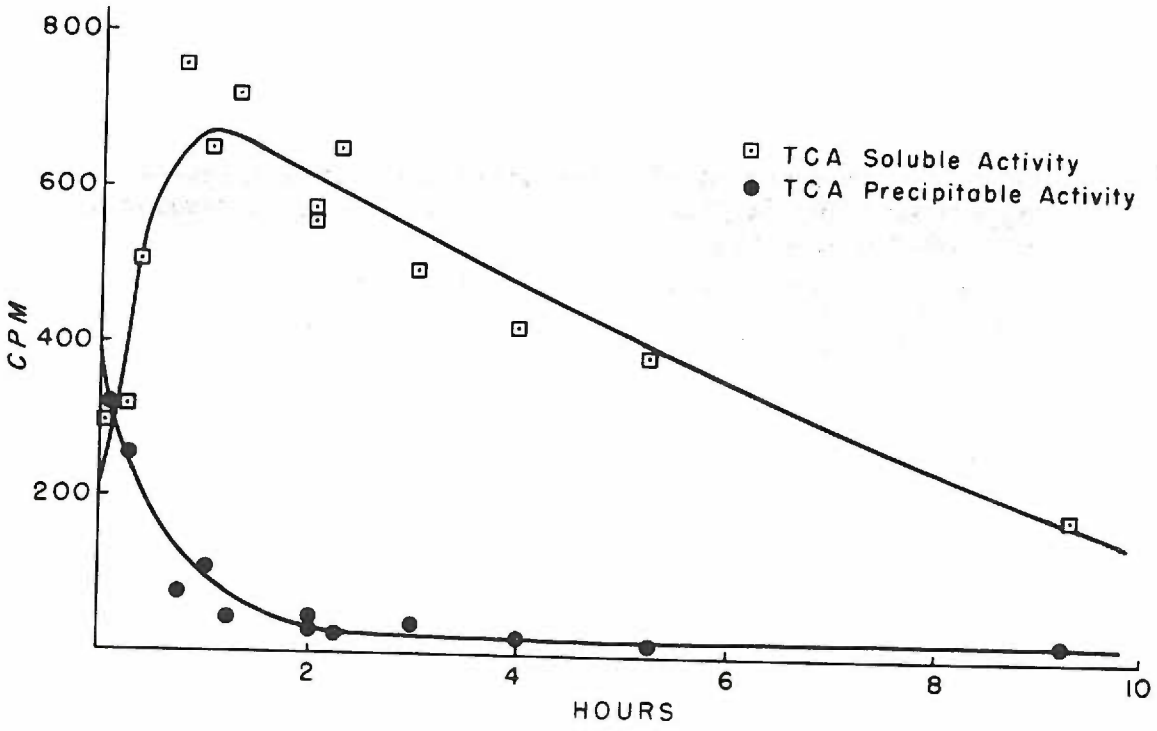
800
600
400
200
CPM
2
4
6
8
10
HOURS
TCA Soluble Activity
TCA Precipitable Activity

Figure 9. Time course of trichloroacetic acid precipitable activity in the aqueous following intravenous injection of I^{131} labeled insulin.

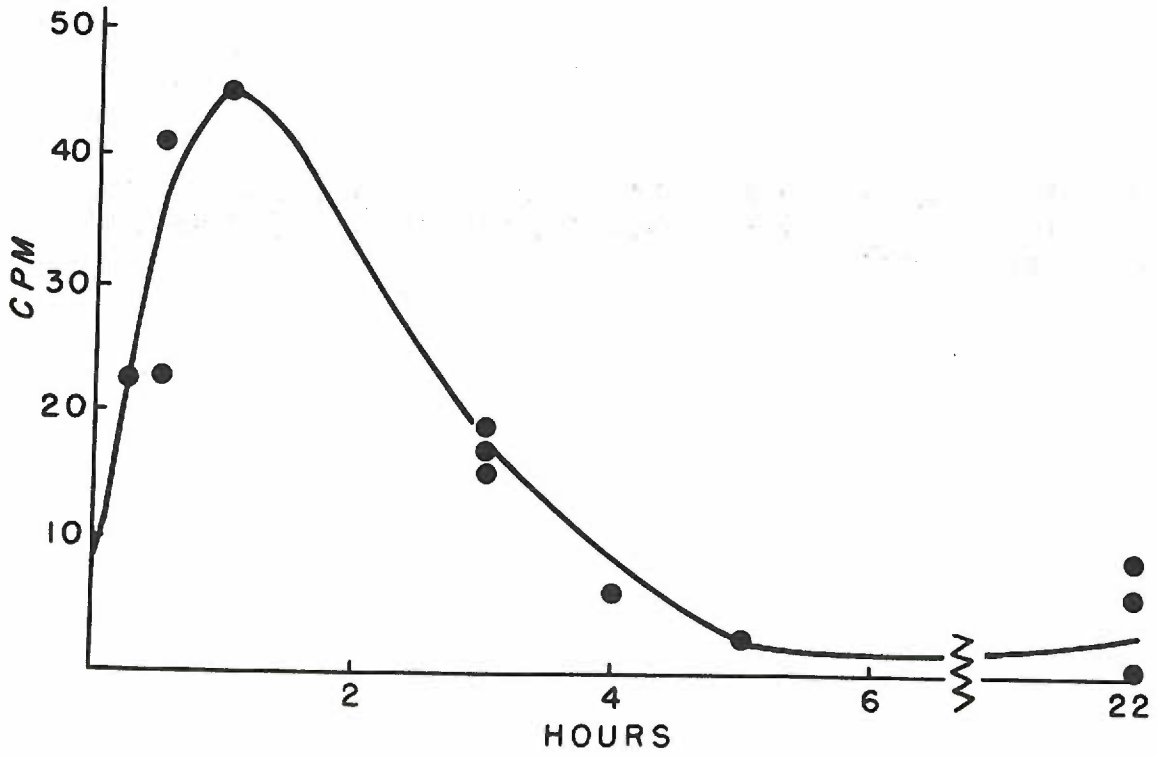
50
40
30
20
10
CPM
2
4
6
22
HOURS

Figure 10. Radioelectrophoretograms of labeled insulin under varied conditions.

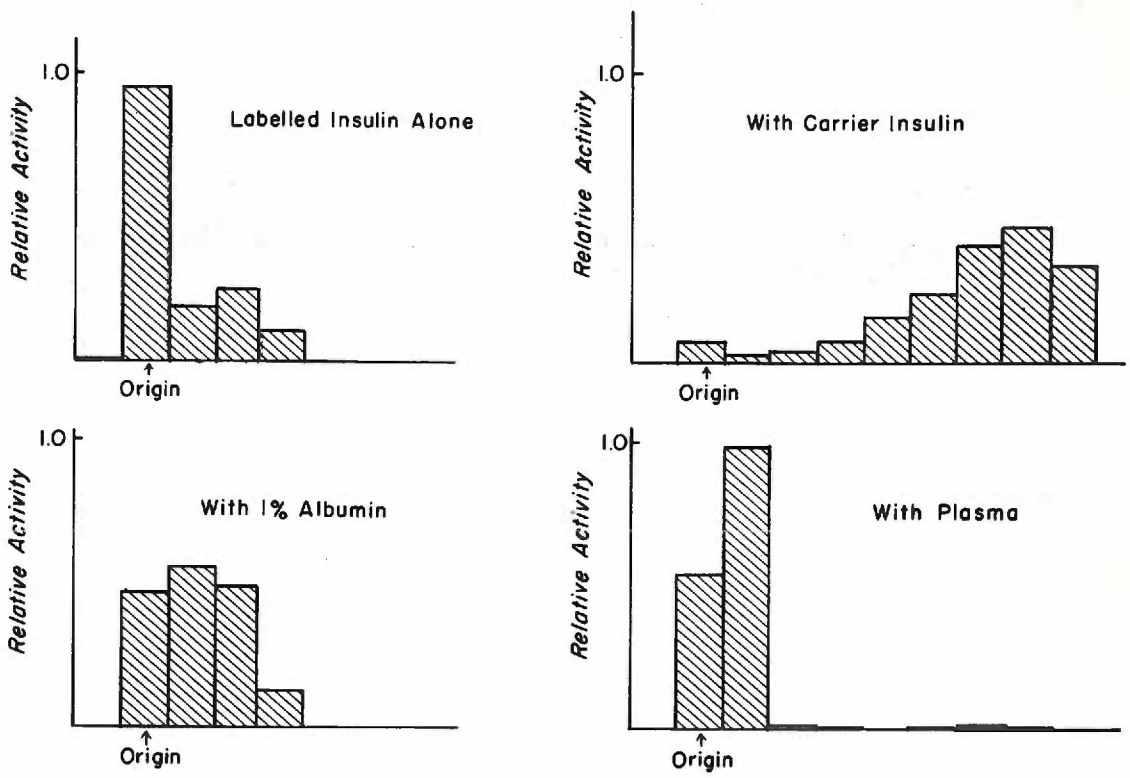
1.0
Relative Activity
Labelled Insulin Alone
Origin
1.0
Relative Activity
With Carrier Insulin
Origin
1.0
Relative Activity
With 1% Albumin
Origin
1.0
Relative Activity
With Plasma
Origin

Figure 11. Radioelectropherotograms of plasma from normal rabbits at progressive time periods following the intravenous injection of I^{131} labeled insulin. "Insulin" activity is that activity represented by the second and third bars. Protein activity is the activity under all bars. Total counts refers to all activity on strip before electrophoresis.

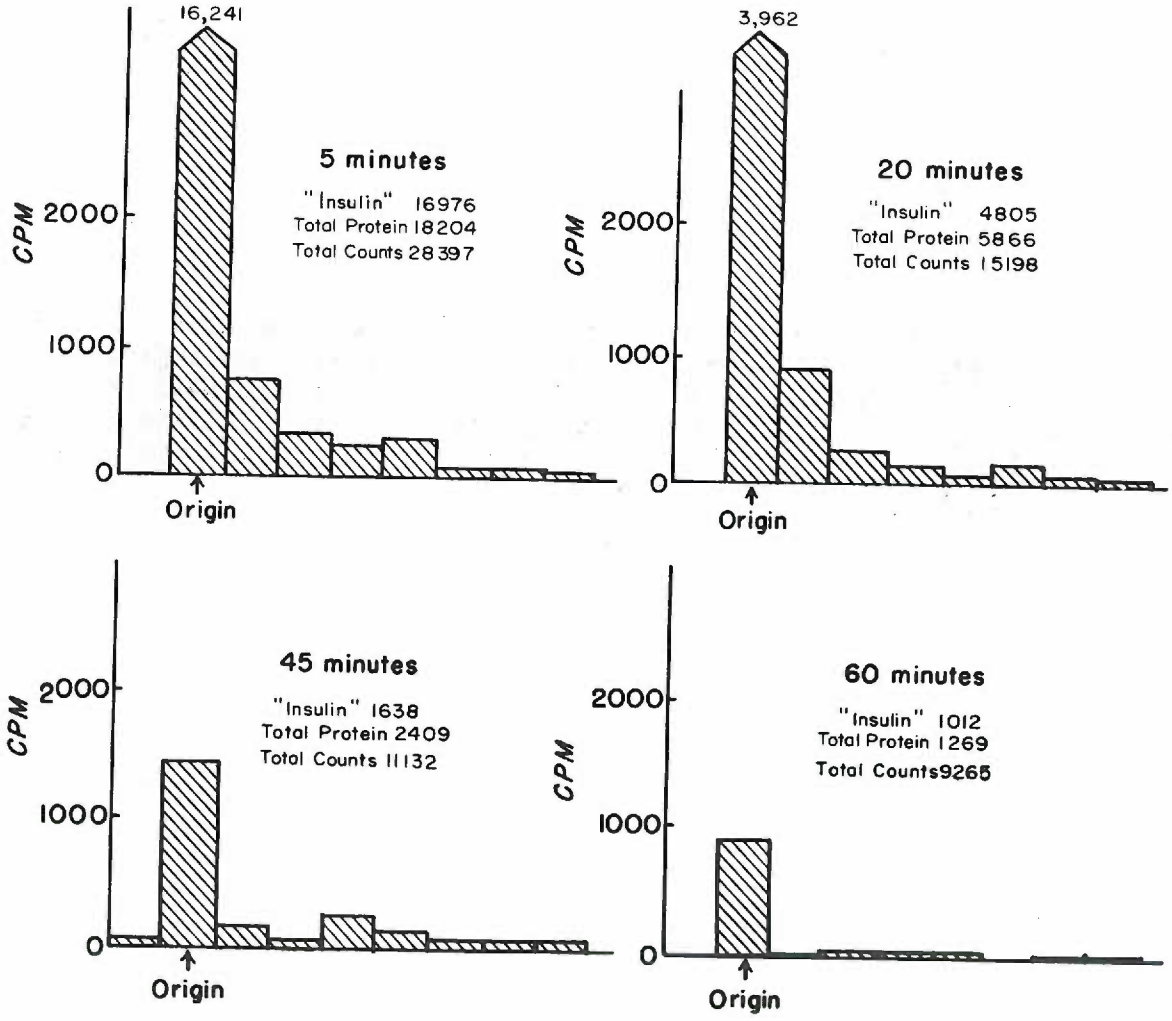
16,241
5 minutes
"Insulin" 16976
Total Protein 18204
Total Counts 28397
CPM
2000
1000
0
Origin
3,962
20 minutes
"Insulin" 4805
Total Protein 5866
Total Counts 15198
CPM
2000
1000
0
Origin
45 minutes
"Insulin" 1638
Total Protein 2409
Total Counts 11132
CPM
2000
1000
0
Origin
60 minutes
"Insulin" 1012
Total Protein 1269
Total Counts9265
CPM
2000
1000
0
Origin

Figure 12. Radioelectrophoretograms of aqueous from normal rabbits at progressive time periods following the intravenous injection of I^{131} labeled insulin. "Insulin" activity is that activity represented by the second and third bars. Protein activity is that represented by all bars. Total counts refers to all activity on the strip before electrophoresis.

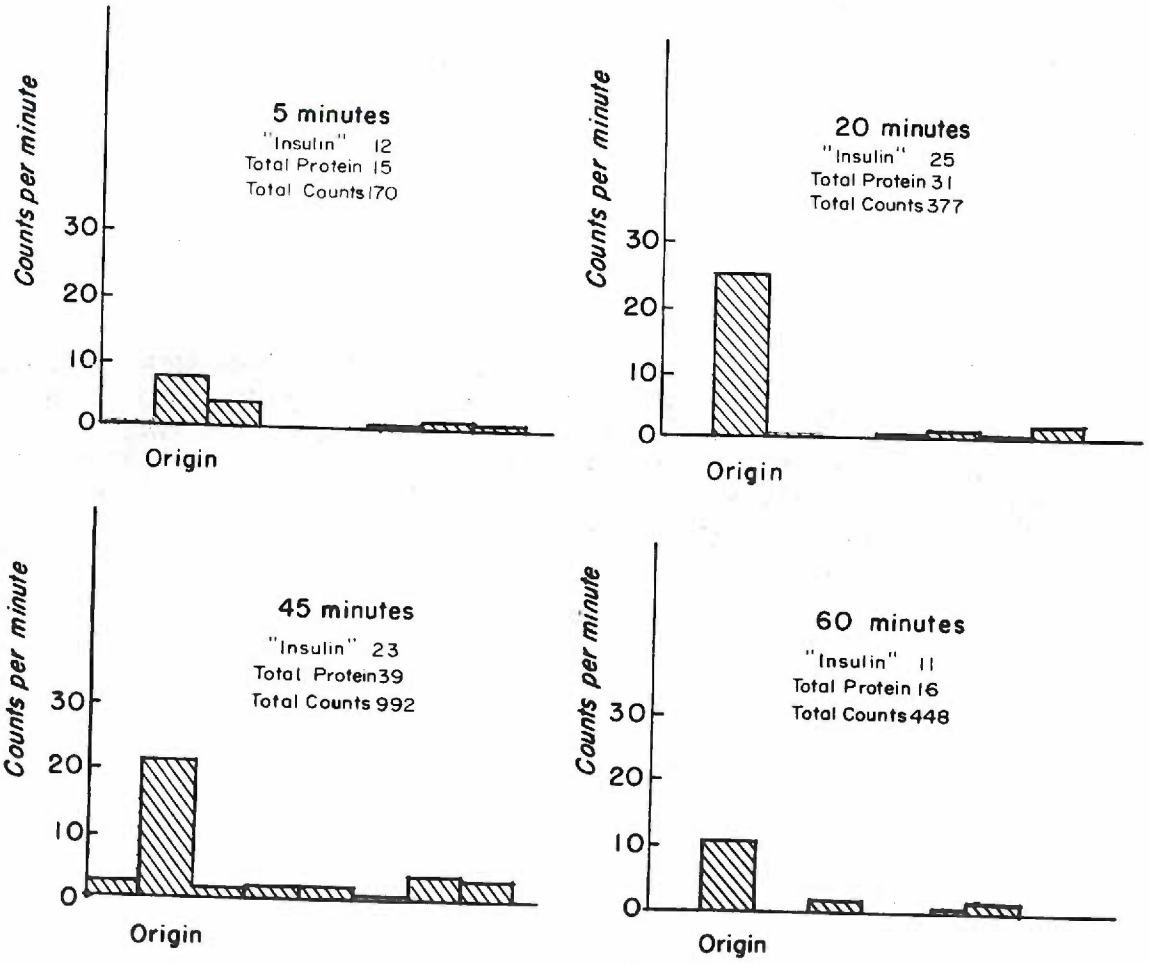
Counts per minute
5 minutes
"Insulin" 12
Total Protein 15
Total Counts 170
30
20
10
0
Origin
Counts per minute
20 minutes
"Insulin" 25
Total Protein 31
Total Counts 377
30
20
10
0
Origin
Counts per minute
45 minutes
"Insulin" 23
Total Protein 39
Total Counts 992
30
20
10
0
Origin
Counts per minute
60 minutes
"Insulin" 11
Total Protein 16
Total Counts 448
30
20
10
0
Origin

Figure 13. Radioelectrophoretograms of aqueous and aqueous plus carrier insulin following intravenous injection of I^{131} labeled insulin into normal rabbits.

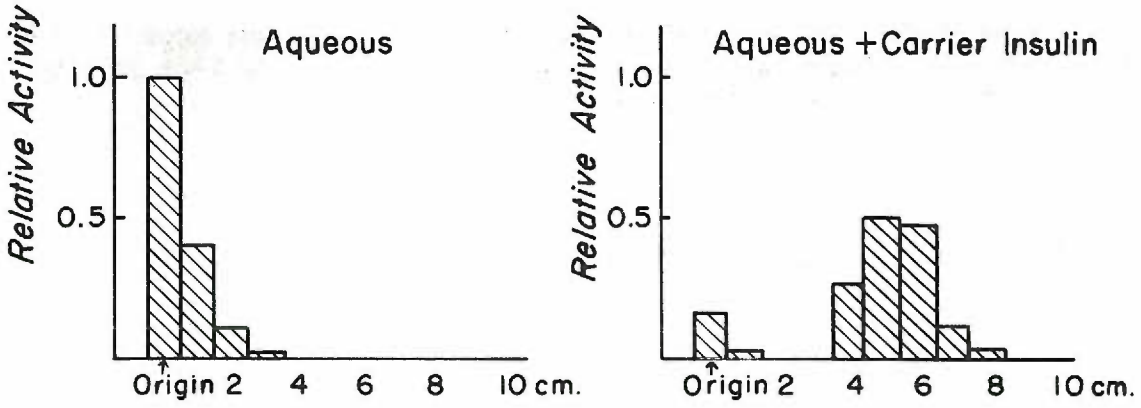
Relative Activity
Aqueous
1.0
0.5
Origin 2 4 6 8 10 cm.
Relative Activity
Aqueous + Carrier Insulin
1.0
0.5
Origin 2 4 6 8 10 cm.

Figure 14. Time course of activity considered to be insulin in the plasma and aqueous of normal rabbits following the intravenous administration of I^{131} labeled insulin.

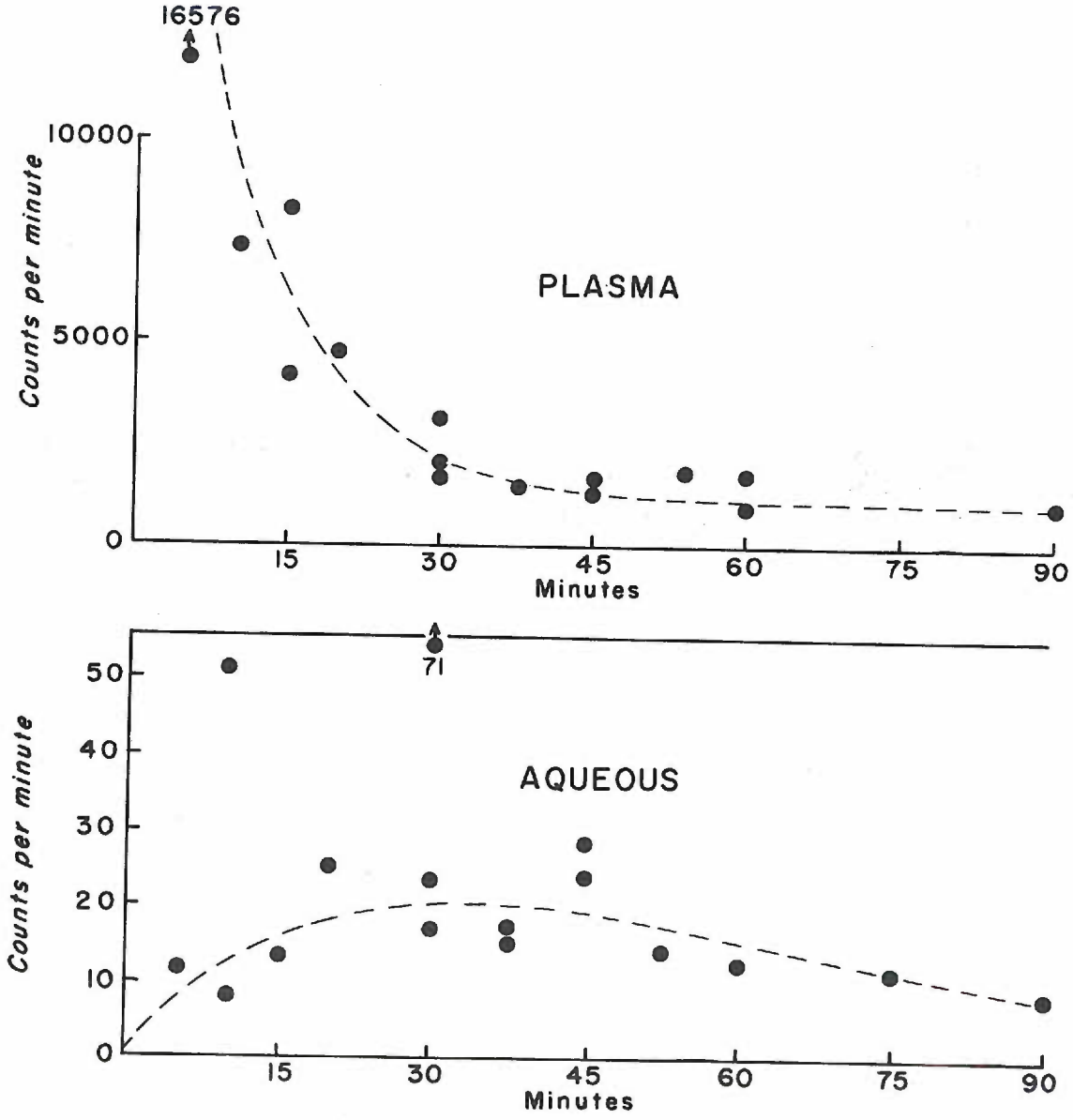
16576
10000
5000
0
Counts per minute
PLASMA
15
30
45
60
75
90
Minutes
71
50
40
30
20
10
0
Counts per minute
AQUEOUS
15
30
45
60
75
90
Minutes

Figure 15. Time course of activity considered to be insulin in the plasma and aqueous of diabetic rabbits following the intravenous administration of I^{131} labeled insulin.

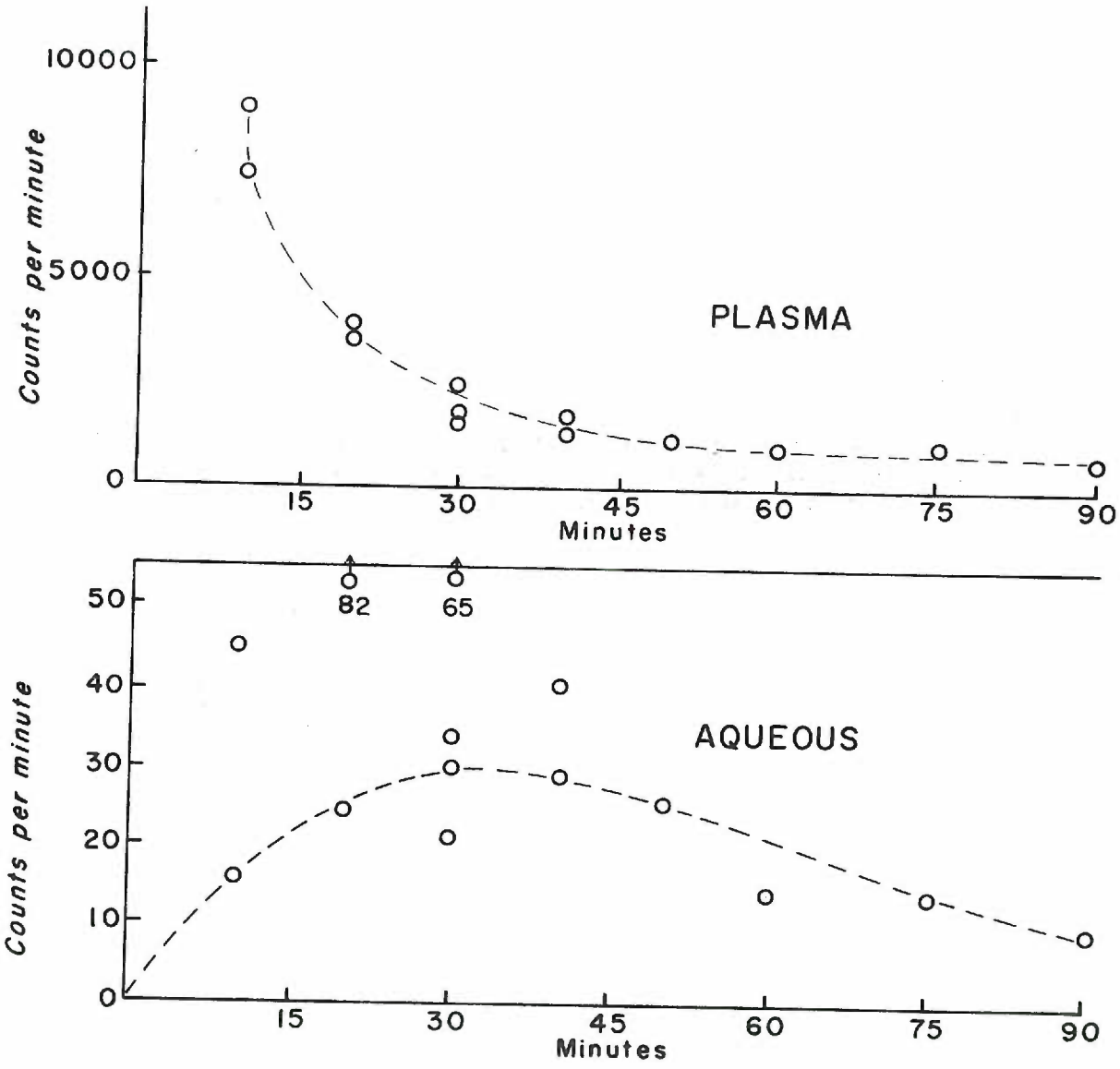
10000
5000
0
Counts per minute
PLASMA
15
30
45
60
75
90
Minutes
50
40
30
20
10
0
82
65
AQUEOUS
Counts per minute
15
30
45
60
75
90
Minutes

Figure 16. Time course of activity considered to be insulin in the plasma and aqueous of rabbits with exogenous hyperglycemia following the intravenous administration of I^{131} labeled insulin.

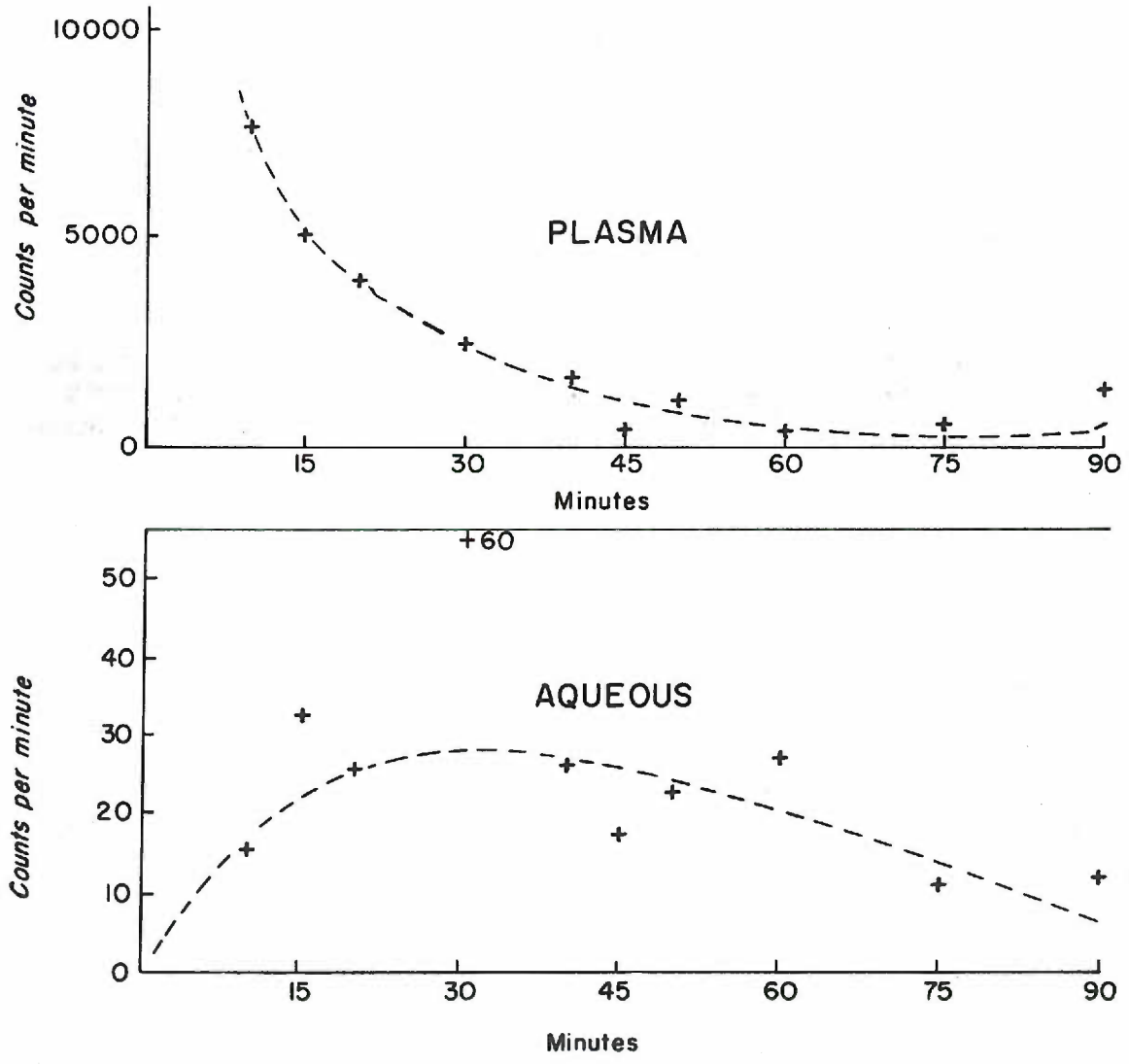
10000
5000
0
Counts per minute
PLASMA
15
30
45
60
75
90
Minutes
+60
50
40
30
20
10
0
Counts per minute
AQUEOUS
15
30
45
60
75
90
Minutes

Figure 17. Composite of activity considered to be insulin in the plasma of rabbits under varied conditions following the intravenous administration of I^{131} labeled insulin.

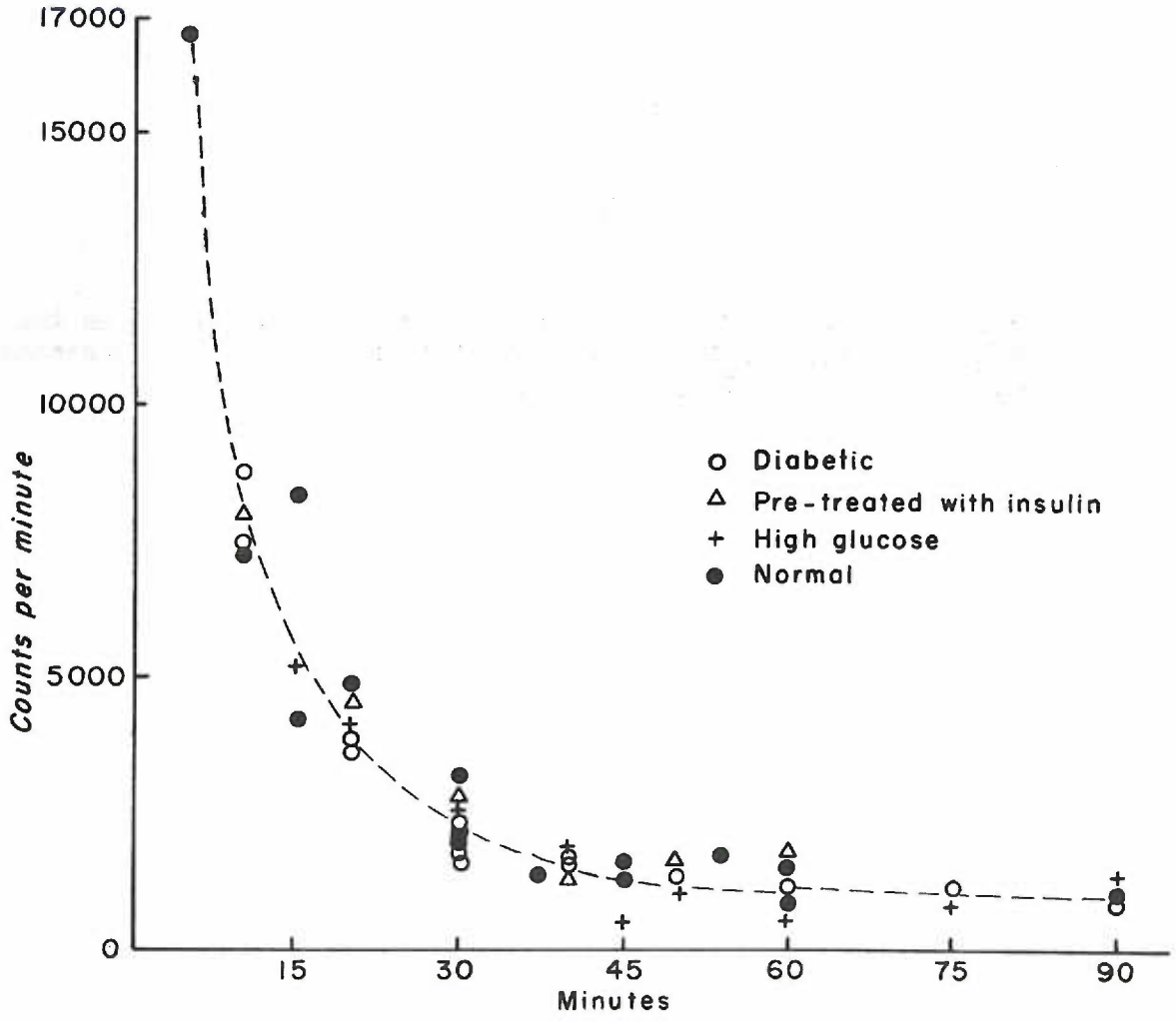
17000
15000
10000
5000
0
Counts per minute
15
30
45
60
75
90
Minutes
Diabetic
Pre-treated with insulin
High glucose
Normal

Figure 18. Composite of activity considered to be insulin in the aqueous of rabbits under varied conditions following the intravenous administration of I^{131} labeled insulin.

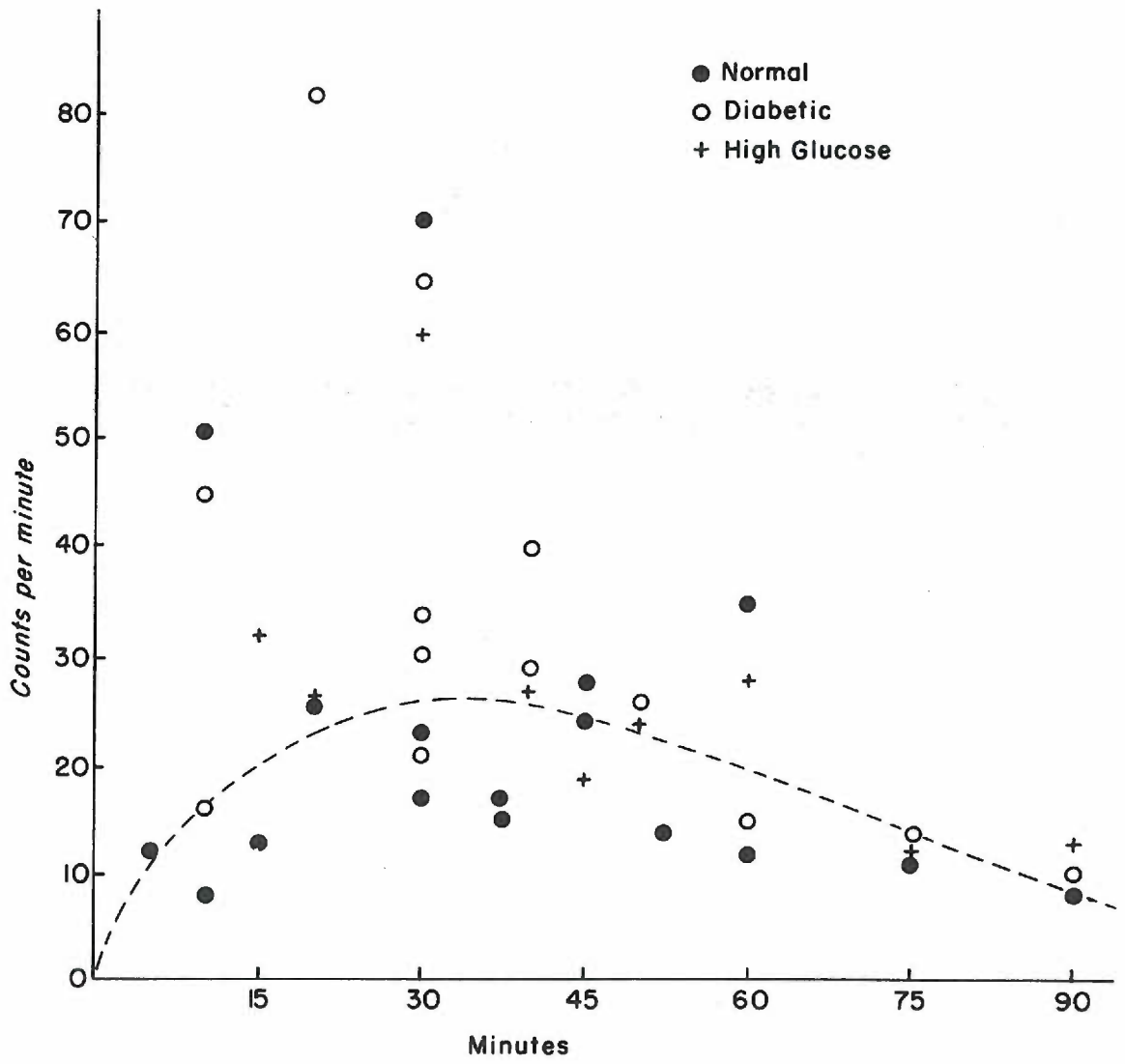
Normal
Diabetic
High Glucose
Counts per minute
0
10
20
30
40
50
60
70
80
15
30
45
60
75
90
Minutes

Figure 19. Comparison of the radioelectrophoretic patterns of plasma from rabbits under varied conditions drawn 15 minutes following the intravenous administration of I^{131} labeled insulin.

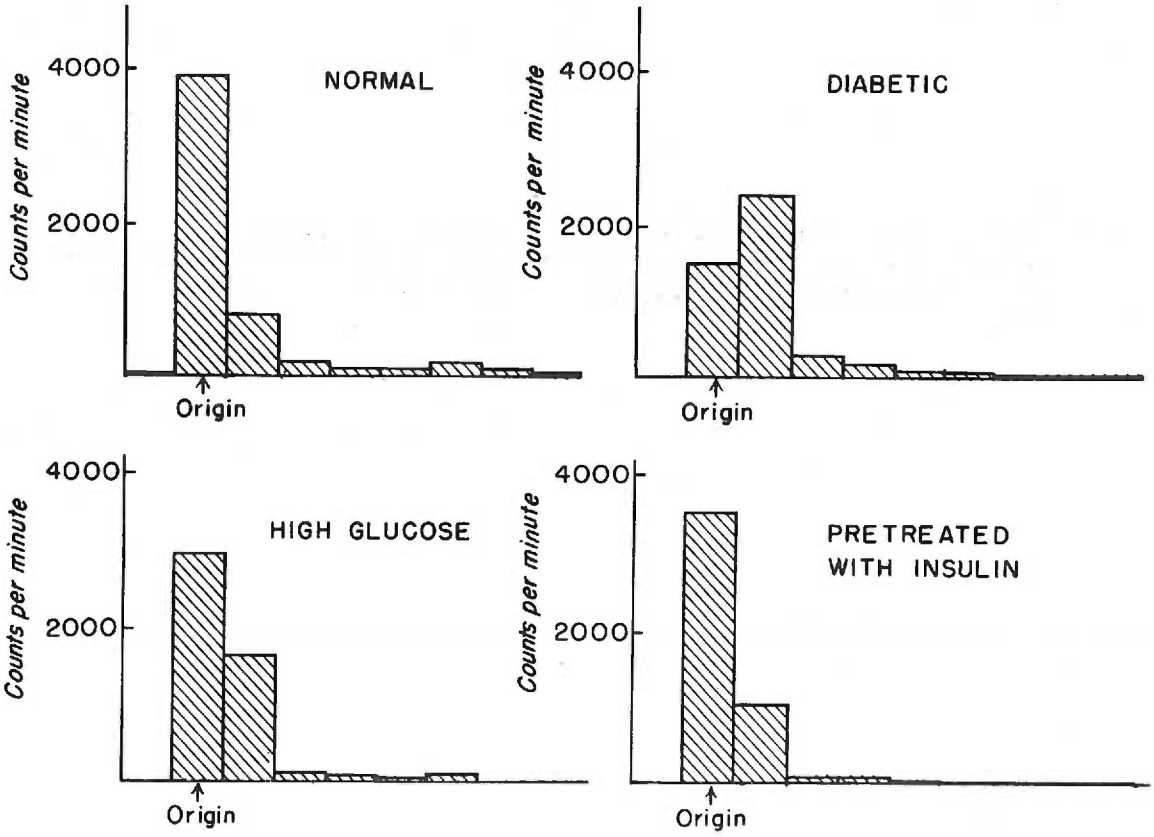
NORMAL
DIABETIC
HIGH GLUCOSE
PRETREATED
WITH INSULIN
Counts per minute
4000
2000
Origin

Table 1. Composition of the basic incubation medium.

TABLE 1

Components	Mgm/100 cc.
Glucose	200.0
NaCl	689.8
KCl	38.0
$CaCl_2 \cdot 2H_2O$	10.0
$MgCl_2 \cdot 6H_2O$	26.5
$NaH_2PO_4 \cdot H_2O$	2.5
$NaHCO_3$	250.0

Na^+ concentration....148 meq/L.

K^+ concentration..... 5 meq/L.

HCO_3^- concentration....30 meq/L.

Table 2. Separation of accumulated activity into three basic fractions.

TABLE 2

Rabbit no.	1	2	3	4
Activity (CPM) under glucose	559	445	658	574
Other activity (CPM) on chromatography strip	153	156	183	128
Activity (CPM) in residual fraction	235	176	227	144
Total activity (CPM) in both lenses	947	777	1068	846
Per cent of activity under glucose spot	59.2	57.3	64.4	67.8