THE EFFECT OF INSULIN, ALLOXAN DIABETES, AND ANOXIA ON THE ULTRASTRUCTURE OF THE RAT HEART

DAVID N. ORTH and HOWARD E. MORGAN

From the Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee. Dr. Orth's present address is Department of Medicine, The Johns Hopkins Hospital, Baltimore. Dr. Morgan is an Investigator, Howard Hughes Medical Institute

ABSTRACT

Hearts from normal and alloxan diabetic rats were perfused *in vitro* with a bicarbonate-buffered medium containing glucose. Transport of glucose through the cell membrane was stimulated with insulin or by induction of anaerobiosis. The organs were rapidly fixed and examined by electron microscopy. Transport stimulation was not associated with any increase in the number of sarcolemmal invaginations or subsarcolemmal cytoplasmic vesicles. It was concluded that glucose transport and the effects of insulin or anoxia do not involve pinocytosis. The relationship of pinocytosis to glucose transport is discussed. The appearance of numerous lipid inclusions at the Z line level of the sarcomeres in the diabetic and anoxic myocardia is described.

Insulin stimulates the uptake of a variety of apparently unrelated substances by the isolated perfused rat heart. In this regard, membrane transport of glucose and monosaccharides (1, 2) and the non-metabolized amino acid, α-amino-isobutyric acid (3), are stimulated by the hormone. In addition, insulin has been observed to increase the uptake of inorganic phosphate and potassium (4). The effects on amino acid and ions have been observed in glucose-free buffer and are, therefore, assumed to be independent of the stimulation of glucose transport.

The monosaccharide transport system in this tissue has the characteristics of "carrier" transport by facilitated diffusion (2). These properties are retained during stimulation of transport by insulin. α-Amino-isobutyric acid is transported against a concentration gradient, indicating that this substance penetrates by "active" transport. Since insulin alters permeability of the membrane to substances penetrating by different mechanisms, the hormone would appear to exert its effect on a membrane property common to the penetration of these substances, *e.g.*, membrane structure.

Insulin stimulates glucose uptake by the rat epididymal fat pad (5). It has been inferred by analogy with other tissues that this effect is due to acceleration of membrane transport, although no direct measurements of transport have been made. Barrnett and Ball (6–8) have shown by electron microscopy that the hormone apparently induces formation of numerous invaginations of the plasma membrane and increased vesiculation of the underlying cytoplasm in adipose cells. This change in membrane morphology suggests a hormone stimulation of pinocytosis, which in itself may lead to a more rapid entry of glucose into the cell. Insulin has also been observed to stimulate pinocytosis in HeLa cells (9), but whether or not this effect is associated with increased glucose transport is not known.

It appeared of interest, therefore, to determine

TABLE I

Effect of Insulin, Anoxia, and Alloxan Diabetes on the Number of Invaginations and Vesicles Associated with the Sarcolemma

Group	Conditions of perfusion	Insulin added	Invaginations per micron of sarcolemma	Vesicles per micron of sarcolemma
Normal hearts				
1	10 ml washout	No	0.48 ± 0.01	3.5 ± 0.2
2	Aerobic, no glucose	No	0.45* ± 0.02	3.5* ± 0.2
3	Aerobic, glucose	No	0.54*† ± 0.02	3.5* ± 0.2
4	Aerobic, glucose	Yes	0.49* ± 0.05	3.0* ± 0.2
5	Anaerobic, glucose	No	0.49* ± 0.04	3.1* ± 0.2
Diabetic hearts				
6	Aerobic, glucose	No	0.49* ± 0.02	3.4* ± 0.2
7	Aerobic, glucose	Yes	0.53* ± 0.04	3.3* ± 0.1

Three hearts were perfused in each group as described under "Methods." A quantitative estimate of the number of vesicles immediately subjacent to a micron of sarcolemma and the number of invaginations per micron of sarcolemma was made for each tissue using a print magnification of × 42,000. An average of 350 microns of sarcolemma was examined in each heart. The means and standard errors of vesicles and invaginations for each group are listed in the table.

*$p > 0.05$ *vs.* group 1.

† $p = 0.05$ *vs.* group 2.

whether insulin would induce morphological changes indicative of pinocytosis in myocardium under conditions in which accelerated transport was known to occur. With this in mind, hearts from normal and alloxan diabetic rats have been perfused with insulin and examined with the light and electron microscopes. Since anoxia has also been shown to cause an acceleration of transport (10), the possible association of this effect with pinocytosis has been examined. In hearts from

FIGURE 1

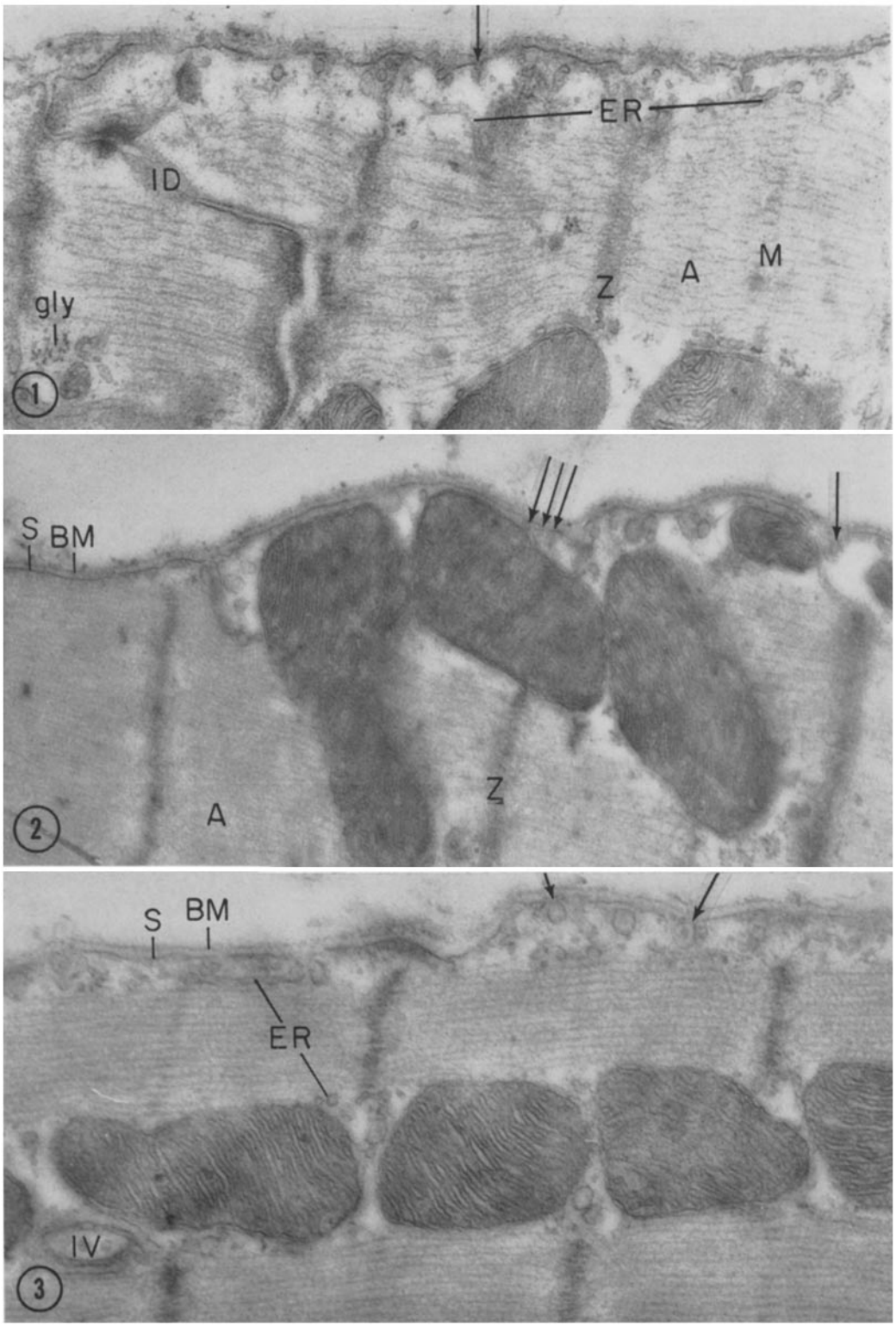
Longitudinal section at the edge of a cell from group 1 (normal, 10 ml washout) showing the sarcolemma, a sarcolemmal invagination (arrow), subsarcolemmal vesicles in close proximity to elements of the endoplasmic reticulum (*ER*), and part of an intercalated disc (*ID*). The contractile apparatus is in a state of contraction so that only the A band (*A*) is demonstrated. The Z line (*Z*) and M band (*M*) are visible, and a small collection of "granules," probably glycogen (*Gly*), are seen in the interstices between the myofilaments. The count in this 3.3 micron length of sarcolemma is two invaginations, twenty-two vesicles. × 42,000.

FIGURE 2

A similar section of a cell from group 2 (normal, 15 minute aerobic perfusion without glucose or insulin). The sarcolemma (*S*) and investing basement membrane (*BM*) are shown, with four mitochondria lying beneath them. Four invaginations (arrows) and fourteen vesicles are present. *A*, A Band; *Z*, Z line. × 42,000.

FIGURE 3

A longitudinal section of the edge of a cell from group 3 (normal, perfused for 15 minutes with glucose, without insulin). The sarcolemma (*S*) and basement membrane (*BM*) are similar to those in the previous figures. The endoplasmic reticulum (*ER*) fills the interstices between the myofilaments and is closely associated with the sarcolemma. A profile of a transverse tubular element (*IV*) is shown. × 42,000.


ER
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1
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IV
3

diabetic rats and in normal hearts exposed briefly to anoxia, the appearance of numerous lipid inclusions is described.

MATERIALS AND METHODS

Animals

Hearts were removed from male albino Sprague-Dawley rats weighing 200 to 300 gm. The animals were fed a stock laboratory diet and had free access to water at all times. Food was withheld for 18 to 20 hours prior to each experiment. Diabetes was induced by the intravenous injection of alloxan, 60 mg per kg, 2 to 4 days before sacrifice. The animals were not heparinized. The fasting plasma glucose of the diabetic animals ranged from 484 to 842 mg per 100 ml.

Perfusion Apparatus

The heart perfusion apparatus has been described elsewhere (11).

Perfusion Medium

The medium was Krebs bicarbonate buffer (12) maintained at 37°C and pH 7.4. The gas phase was 95 per cent O_2 plus 5 per cent CO_2 except for the anaerobic perfusions, in which O_2 was replaced with N_2. Insulin was added where indicated in a concentration of 3 μg per ml (0.1 unit per ml) and glucose in a concentration of 400 mg per 100 ml. The hormone (kindly supplied by Eli Lilly and Co., lot no. 466367) had been treated to remove glucagon.

Procedure

The rats were decapitated and the heart was excised and placed in physiological saline at 4°C as described earlier (11). Since no heparin was used, speed was essential, and no more than 15 seconds elapsed between the death of the animal and cessation of cardiac contractions in the cold medium. The heart was then attached by the aorta to a cannula and perfusion was begun with warm medium. The first 10 ml of buffer, which washed out the blood in the vascular system, was discarded. The remaining 20 ml of medium was recirculated through the organ with continuous gassing. The period from decapitation to resumption of muscular contractions in the warm medium was about 80 seconds, during most of which time the heart was protected from anoxia by the low temperature. Perfusions were continued for 15 minutes. All the aerobically perfused hearts were observed to beat at normal rates, while those perfused anaerobically slowed, became arrhythmic, and finally stopped.

Preparation for Electron Microscopy

The heart preparation permits extremely rapid fixation of the tissue. At the conclusion of the perfusion period, a no. 22 needle was inserted into the tubing just above the aortic cannula. The tubing was clamped above the needle and 2 ml of the fixative, 1 per cent osmium tetroxide buffered with veronal-

FIGURE 4

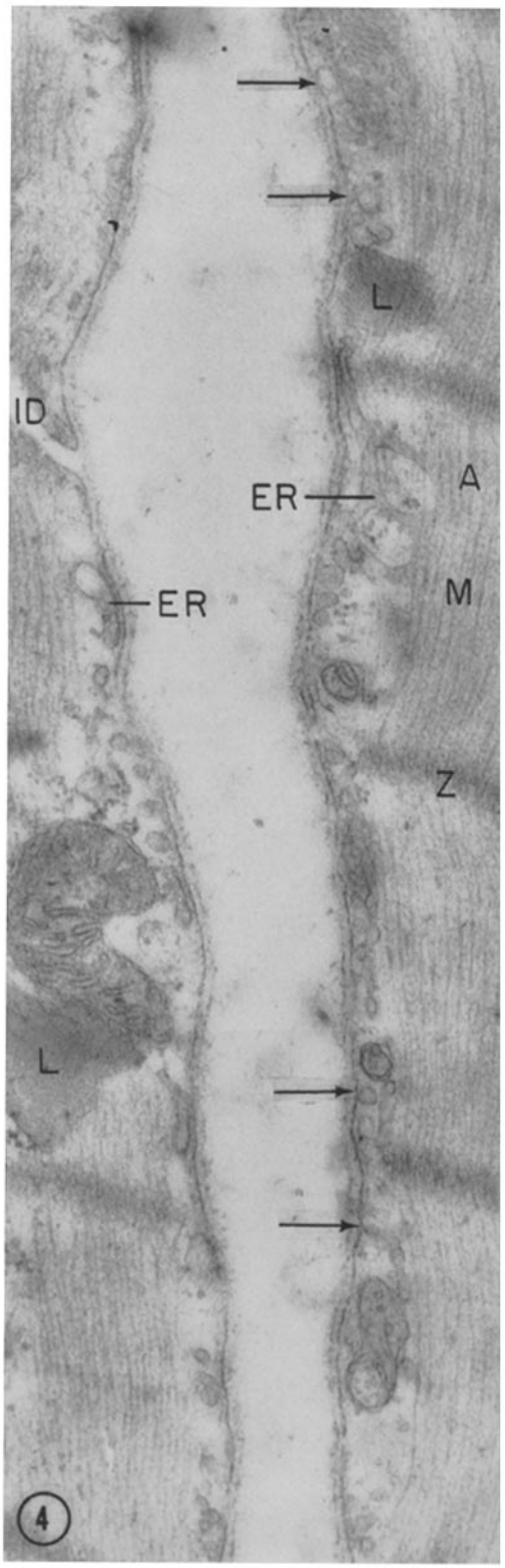
A longitudinal section of the edges of two adjacent cells from group 6 (diabetic, perfused for 15 minutes with glucose, without insulin). Part of an intercalated disc (*ID*) is seen in the cell on the left, and in both cells elements of the endoplasmic reticulum (*ER*) lie in intimate association with the sarcolemma and the subsarcolemmal vesicles, of which thirty-nine are present beneath the 10 microns of sarcolemma visible here. Two small lipid inclusions (*L*) are located adjacent to the Z lines (*Z*), and four invaginations are indicated by the arrows. *A*, A band; *M*, M line. × 42,000.

FIGURE 5

A longitudinal section of a cell from group 3 (normal, 15 minute perfusion with glucose, without insulin) demonstrating the morphology of the occasional lipid inclusion (*L*) seen in the normal tissue and its intimate relationship with the mitochondrion. Longitudinal elements of the endoplasmic reticulum (*ER*) are tangentially sectioned. *M*, M line; *Z*, Z line. × 42,000.

FIGURE 6

A section of a diabetic heart (group 6) which demonstrates the typical morphology of the lipid inclusions (*L*) and their location at, or tangent to, the Z lines (*Z*). The inclusions lie in proximity to apparently normal elements of the endoplasmic reticulum (*ER*) and the transverse tubular system (*IV*). × 42,000.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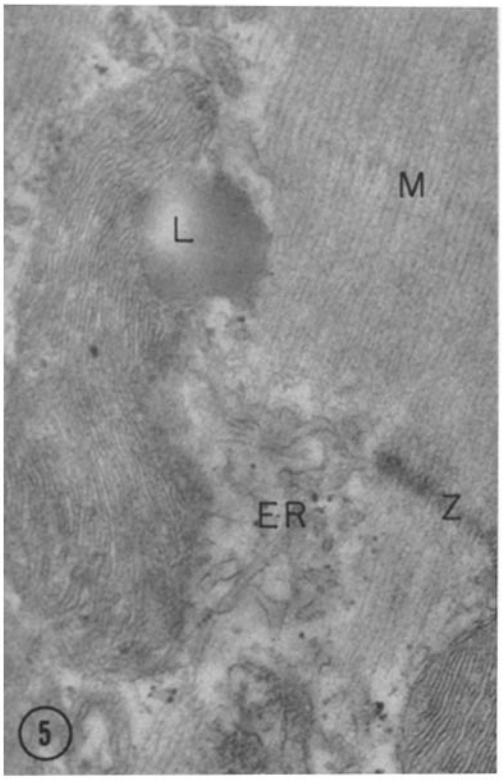
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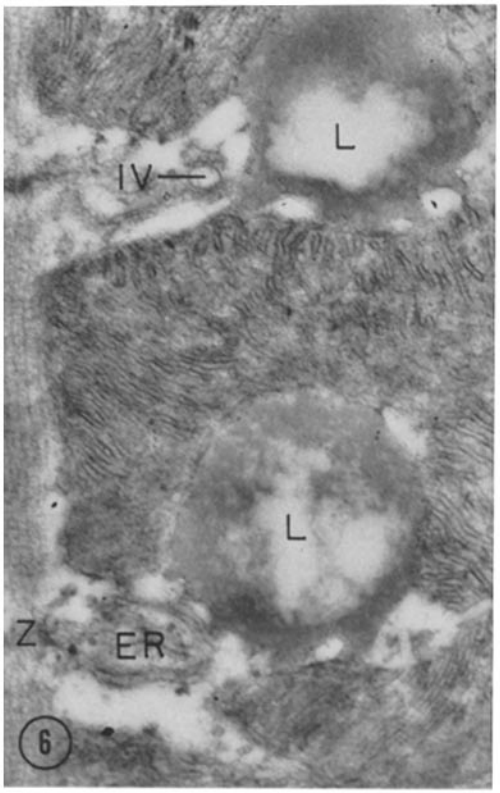
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6

acetate to pH 7.4, made isosmolar with sucrose and cooled to about 4°C, was rapidly injected. The heart stopped beating in systole and immediately turned brown and hardened considerably. Two 1-mm-thick midventricular transverse sections were cut using a device holding three parallel razor blades. These sections were then cut into 1 mm^3 blocks, which were left in the fixative for 2 hours at 4°C.

Dehydration was carried out at room temperature in increasing concentrations of ethanol from 50 to 100 per cent. The dehydrated blocks were infiltrated and embedded in a 9:1 mixture of Du Pont *n*-butyl and methyl methacrylate with 0.2 per cent benzoyl peroxide as the catalyst. Thin sections, cut with freshly prepared glass knives on a Porter-Blum microtome, were mounted on grids covered with a thin carbon membrane and stained with 1 per cent potassium permanganate for approximately 30 minutes, rinsed in very dilute citric acid and distilled water (13), and sandwiched with a collodion film. They were then examined with the RCA EMU-2B electron microscope. The tissue blocks were uniformly well fixed throughout, in contrast to results obtained by diffusion fixation.

Preparation for Light Microscopy

The procedures employed were the same as for electron microscopy except that the fixative was 10 per cent aqueous buffered formalin solution containing 1 per cent calcium chloride at 4°C. Frozen sections were stained with Sudan IV (14) and photographed on Agfa IFF-13 film using a blue-green filter (λ = 450 mμ).

OBSERVATIONS

Electron Microscopy of Normal Aerobic Hearts

The structure of cardiac muscle has been described by numerous previous authors (15–26). Attention will, therefore, be given in this paper only to morphological changes resulting from the special conditions to which the hearts were subjected. The appearances of tissue obtained from normal hearts washed free of blood and of that perfused for 15 minutes either in the presence or in the absence of glucose were indistinguishable (groups 1, 2, 3, Table I; Figs. 1 to 3). A considerable expansion of the interstitial space was seen in all sections, due to perfusion with medium lacking the physiological complement of protein. Measurements of the extracellular space of the heart *in vivo* by the distribution of sorbitol have given values of about 250 μl per gm of wet tissue (27). Similar estimates in the isolated heart under the present conditions have shown the extracellular space to be about 360 μl per gm (11). The interstitial edema occurs in the first few minutes and progresses only very slowly thereafter (11). Most of the sarcomeres were in a state of fixation-induced contraction and, therefore, their I bands were not often visualized (Figs. 1 and 2).

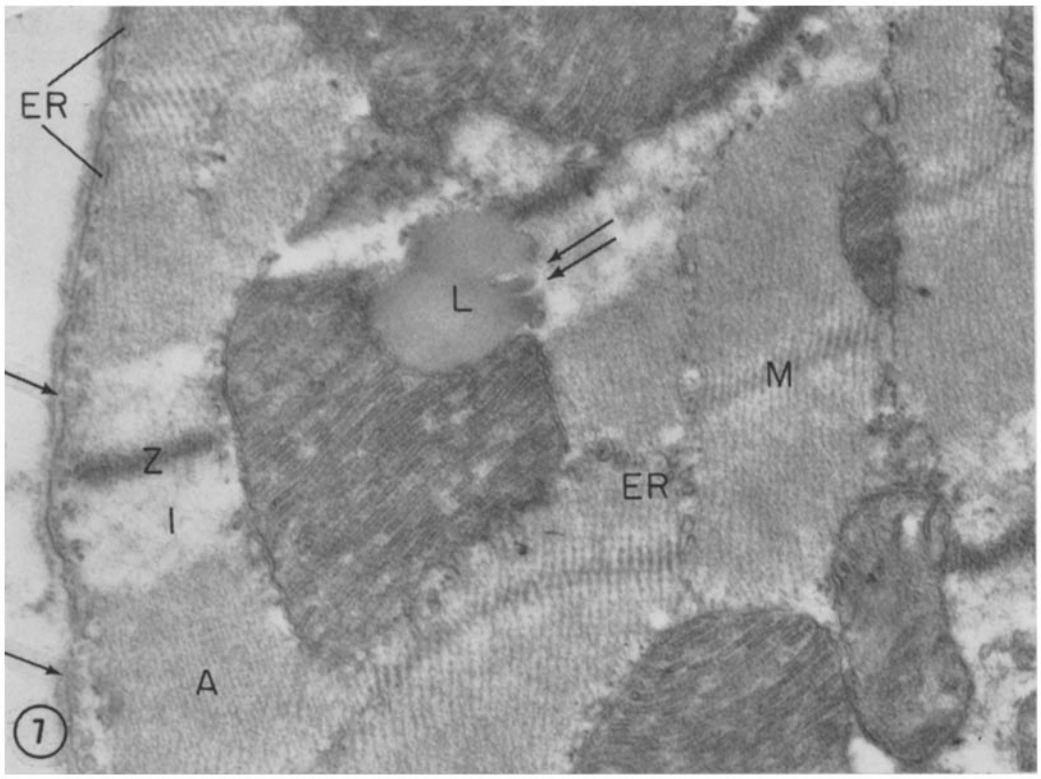
The sarcolemma appeared under all conditions as a homogeneous, continuous, electron-opaque structure 70 to 80 angstroms in width. It

FIGURE 7

A section of a normal cardiac cell anaerobically perfused (group 5) in which the myofilaments are somewhat obliquely sectioned. The A band (*A*), I band (*I*), Z line (*Z*), and M line (*M*) are visible, however. A large mitochondrion embraces a lipid inclusion (*L*) at the Z line level of the sarcomere. The inclusion has the appearance, in some areas, of possessing a limiting membrane which is infolded to form crista-like projections (arrows in center of figure). Longitudinal elements of the endoplasmic reticulum (*ER*) lie immediately below the sarcolemma in close association with fourteen subsarcolemmal vesicles. Two invaginations are indicated by the arrows at the left × 42,000.

FIGURE 8

A similar section from a group 5 tissue. Two intermediary vesicles, or transverse tubular elements, are shown in profile (*IV*). A lipid inclusion (*L*) underlies the sarcolemma and, in the plane of this section, appears not to be tangent to the Z line. Numerous small granules probably representing glycogen (*Gly*) are in close association with elements of the sarcoplasmic reticulum. The sarcolemma is obliquely sectioned here, and an accurate count of vesicles and invaginations is not possible. *M*, M line. × 42,000.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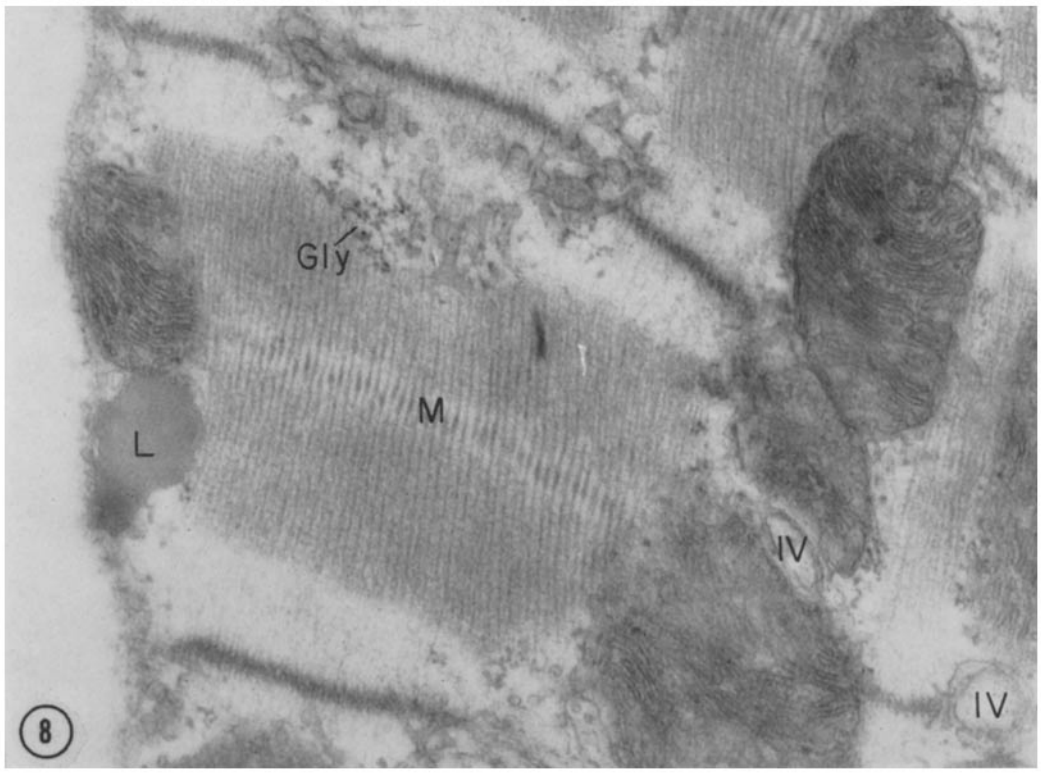
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was invested, where exposed to the interstitial space, with a basement membrane of rather constant width (Figs. 1 to 3). Occasional minute invaginations of the sarcolemma were observed (Table I). Perfusion with glucose produced a suggestive increase in the frequency of invaginations (group 3), otherwise no apparent variations in frequency of invaginations were encountered with the different conditions of perfusion. The cytoplasm subjacent to the sarcolemma contained numerous vesicles whose distribution and frequency were apparently random. While numerous vesicles would be observed underlying one portion of sarcolemma, an adjacent segment might contain few, if any, vesicles (Fig. 2). The diameters of the vesicles and sarcolemmal invaginations, which were bounded by membranes indistinguishable from the sarcolemma itself, ranged from 200 to 600 angstroms. The ubiquitous profiles of the sarcoplasmic reticulum, well described by others (24), ranged from the area immediately below the sarcolemma (Fig. 3) to the perinuclear cytoplasm. No definite connections between the sarcolemma and the reticulum were observed. The characteristic regional specialization with respect to the sarcomeres was visualized. There was no apparent alteration in the morphology of any of the elements of the reticulum.

Moderately large dense bodies were scattered sparsely through the cytoplasm of the normal heart cell (Fig. 5) and were taken to be lipid inclusions on the basis of their morphology and electron opacity. Similar structures have been observed in cardiac muscle by other authors (26). The diameter of these was usually 200 to 400 millimicrons, although some bodies were as large as 1 micron in diameter. They were usually nearly round or oval, but were occasionally quite irregular in outline. Rarely an inclusion appeared to be membrane enclosed. They were in close association with the mitochondria with a tendency to localize at the Z line level of the sarcomere (Fig. 5).

Electron Microscopy of Diabetic Hearts

Measurements of sugar transport in diabetic hearts have indicated that the process is inhibited to about 30 per cent of normal and that the inhibition is due to insulin deficiency. Despite this change in transport rate, the frequency of sarcolemmal invaginations and subsarcolemmal vesiculation was observed to be unchanged (Table I). Similarly, no alteration in sarcolemmal morphology was seen (Fig. 4).

The number and size of lipid inclusions were greatly increased in diabetic myocardium (Fig. 6). These inclusions were located in the columns of mitochondria between the myofibrils either

FIGURE 9

A normal tissue perfused for 15 minutes with glucose and insulin (group 4), showing the apparent lack of increased pinocytotic activity. A single invagination (arrow) and seventeen vesicles are present. A transverse tubular element (*IV*) is shown in profile. There is no apparent change in the morphology of the sarcolemma or basement membrane. *ER*, endoplasmic reticulum. × 42,000.

FIGURE 10

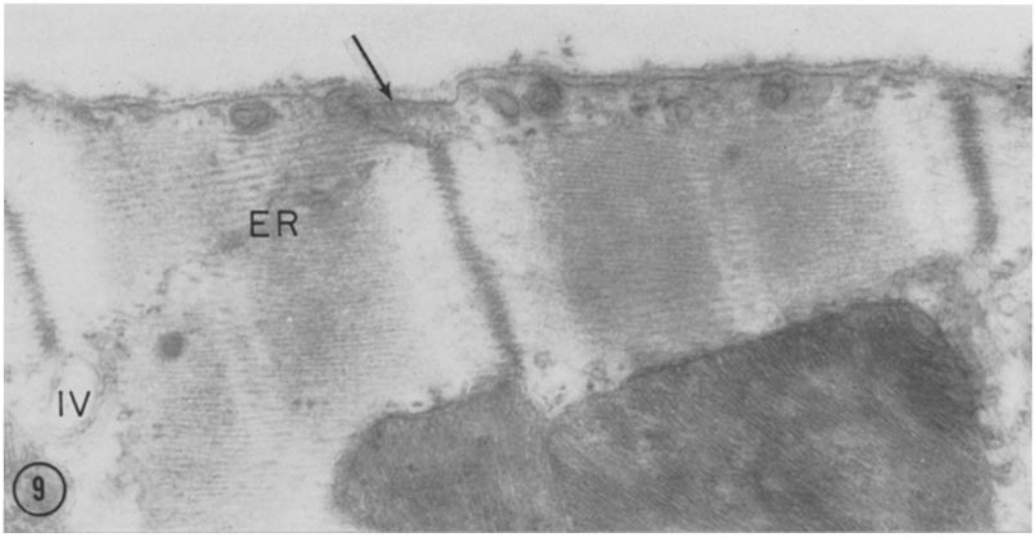
A diabetic tissue perfused with glucose and insulin (group 7), demonstrating the lack of insulin effect on sarcolemmal morphology or pinocytotic activity. Two invaginations (arrows) and twenty vesicles are present. A densely stained lipid inclusion (*L*) underlies the sarcolemma. The attenuated cytoplasm of two adjacent endothelial cells forms a capillary lumen (*Cap*). × 42,000.

FIGURE 11

A normal tissue (group 4) demonstrating the lack of insulin effect on the lipid inclusions (*L*). × 42,000.

FIGURE 12

A similar section from a diabetic tissue (group 7) demonstrating a similar lack of insulin effect on the morphology of the lipid inclusions (*L*). × 42,000.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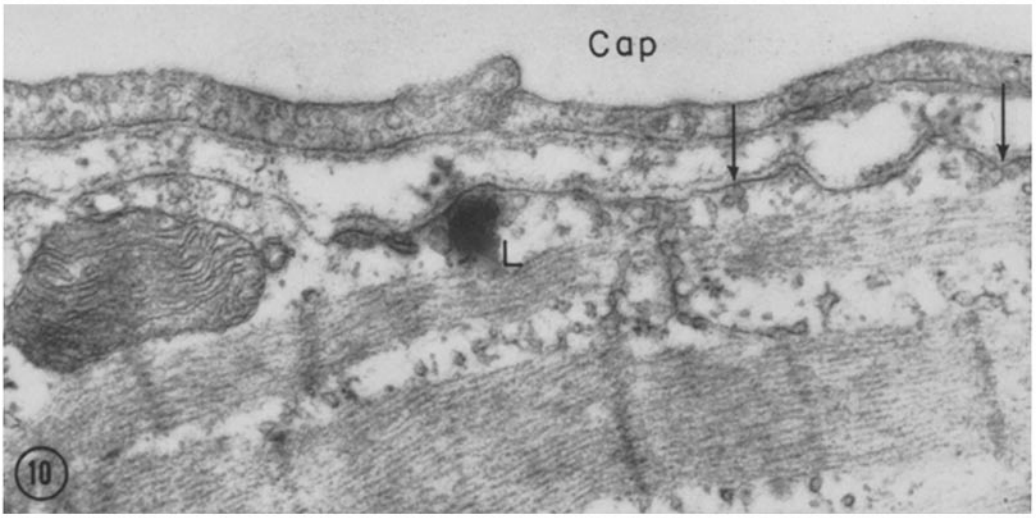
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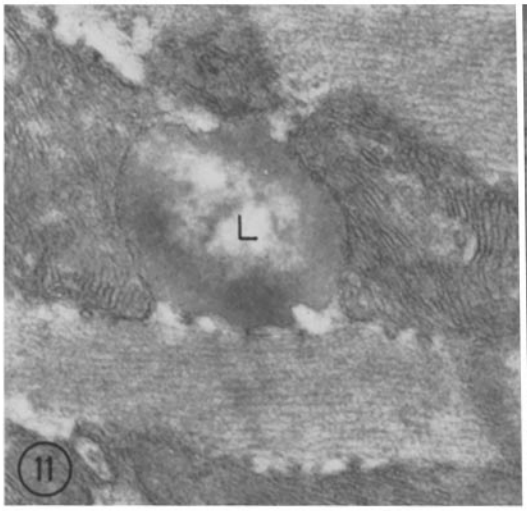
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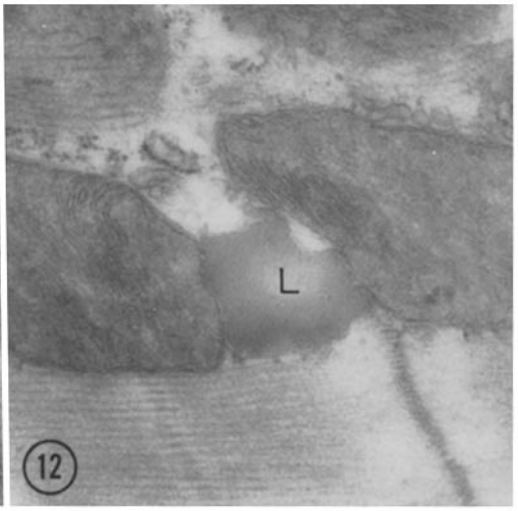
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straddling or, more often, tangent to the Z line of the sarcomere (Fig. 6). While these inclusions were occasionally approximated laterally, they were almost without exception separated from one another longitudinally by mitochondria. This was true even where large inclusions occurred in several consecutive sarcomeres or where, less commonly, two inclusions were found in a single sarcomere. Occasionally the inclusions were completely embraced by a mitochondrion, as described previously in pancreatic and hepatic parenchymal cells (28). These lipid bodies tended to have diameters of 0.7 to 1.0 micron and stained more heavily than their counterparts in normal tissue. Large inclusions often had a clear central area which was thought to be due to incomplete fixation with subsequent elution of lipid during the dehydration process (Fig. 6). In rare instances, the membrane enclosed in the inclusion was observed to have a few infoldings that produced parallel membranes of the same width as mitochondrial cristae (as demonstrated in an anaerobic heart in Fig. 7). These inclusions were occasionally seen in proximity to apparently normal triads of the sarcoplasmic reticulum (Fig. 6).

Effect of Anaerobic Perfusion on the Electron Microscopic Anatomy of the Normal Heart

The general structure of the heart was well maintained during 15 minutes of anaerobic per-

FIGURES 13 TO 19

These are light microscopic sections, fixed in buffered formalin, sectioned on a freezing microtome, and stained with Sudan IV for lipid. They were photographed using a blue-green filter. × 1300.

FIGURE 13

Normal heart, 10 ml washout. There is a complete absence of any lipid material in the cell. Striations are demonstrated.

FIGURE 14

Normal heart, 15 minute perfusion with glucose, as in group 3. Occasional isolated lipid inclusions are seen.

FIGURE 15

Normal heart, perfused anaerobically for 15 minutes with glucose in the buffer, as in group 5. A profusion of small lipid inclusions are well stained and are localized in the interstices between the filament bundles at the Z line level of the sarcomere (arrows).

FIGURE 16

A diabetic heart, 10 ml washout. The inclusions are somewhat larger and more profuse than in the anaerobic tissue, but their localization is similar. Arrow, Z line level.

FIGURE 17

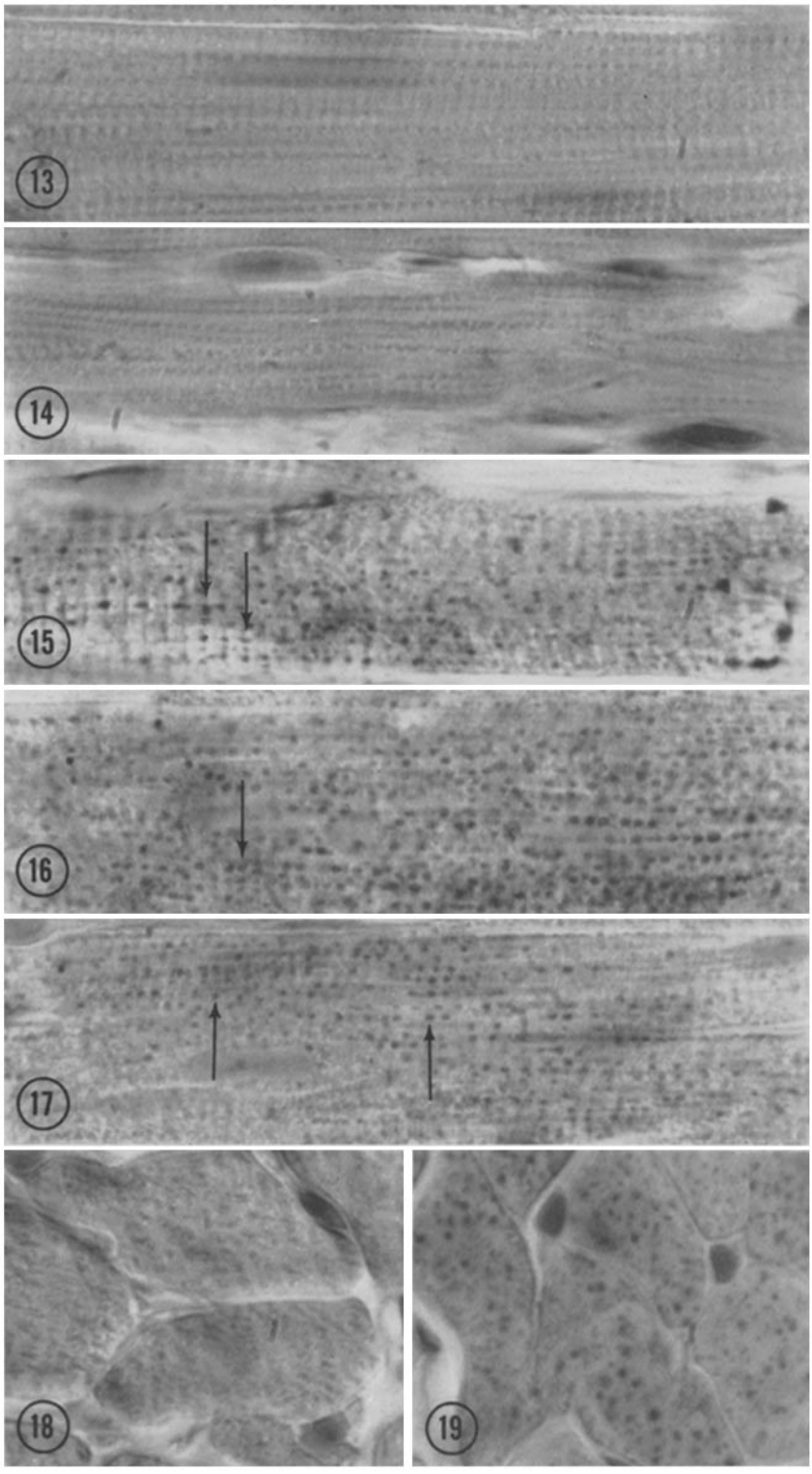
A diabetic heart, perfused for 15 minutes with glucose. Its appearance is essentially the same as that of the diabetic washout. Again, notice the localization at the Z line (arrows).

FIGURE 18

Cross-section of a normal heart, perfused for 15 minutes with glucose. No lipid inclusions are visible.

FIGURE 19

Cross-section of a diabetic tissue, perfused for 15 minutes with glucose in the buffer (as in group 6). Numerous large sudanophilic inclusions are located between the myofilament bundles.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fusion in the presence of glucose (Figs. 7 and 8). The sarcolemma appeared to be intact and the pinocytotic activity unchanged (Table I). The major difference as compared with aerobically perfused hearts was an increase in the number of lipid inclusions. The anatomical and staining appearance of these structures was much the same as that described in relation to diabetic organs, though the frequency and size were less. The mitochondria did not show the swelling previously reported in myocardium made anoxic for longer periods of time (20), but there was some tendency for the intermediary, or transverse, elements of the sarcoplasmic reticulum to swell.

Effect of Insulin on the Electron Microscopic Anatomy of Normal and Diabetic Hearts

Examination of the hormone-treated tissues revealed no changes in any of the components of either tissue. The frequency of invagination and vesiculation was unchanged (Table I; Figs. 9, 10 to 12).

Light Microscopic Appearance of Normal, Diabetic, and Anaerobically Perfused Normal Hearts

All of the organs, when fixed in buffered formalin and stained with hematoxylin and eosin, appeared similar. When stained with Sudan IV, however, essentially no lipid was seen in the normal heart (Figs. 13, 14, and 18). In both the diabetic hearts and those anaerobically perfused, however, numerous sudanophilic particles were observed (Figs. 15 to 17, and 19). These bodies were located within the rows of mitochondria at the Z level and were of the size of the inclusions noted by electron microscopy. Because of their sharp localization, the inclusions sometimes imparted a striking cross-hatched appearance to the myocardium.

DISCUSSION

Earlier studies (11) have shown that insulin or anoxia regularly induces a substantial increase in glucose uptake under the conditions of the present perfusions. Kinetic analysis indicated that membrane transport was depressed by about 60 per cent as a result of alloxan diabetes and that insulin increased transport in the diabetic heart at least 10-fold (29). The hormone, or anoxia, also increased transport several-fold in the normal heart. These stimulations were maintained for periods of at least 30 minutes (11). It could be anticipated, therefore, that increased vesiculation would be evident in the present study if pinocytosis were the mechanism by which insulin or anoxia affected transport. Though no precision can be claimed for the counts of sarcolemmal invaginations and underlying vesicles, a morphological change in any way comparable to the changes in physiological activity should have been readily detectable. That the counts did have considerable reliability was suggested by their surprising constancy from one tissue to the next. The complete absence of any suggestion that insulin induced pinocytosis in the heart is in marked contrast to the finding of Barrnett and Ball (7) in the epididymal fat pad. It is possible, although unlikely, that a faster turnover of vesicles did occur without an increase in their number at any moment.

Some cogent reasons for doubting whether pinocytosis could account for the stimulation of monosaccharide transport have already been given by Barrnett and Ball (7). They calculated that each molecule of insulin would have to induce formation of several thousand pinocytotic vesicles. The imbibition of water in association with glucose uptake would also amount to several times the weight of active cell material within a few minutes, whereas no substantial change in cell water content could be detected. However, these apparent difficulties do not exclude pinocytosis. A single molecule of insulin could be imagined to act catalytically on many molecules of a widespread component in the membrane. The transfer of water out of the cell might be readily accomplished in view of the well known facility and extreme rapidity of diffusion of water through most mammalian cell membranes.

More substantial objections to pinocytosis arise from a consideration of the properties of glucose transport in heart muscle. Transport shows stereospecificity, saturation kinetics, counterflow, and free reversibility with similar kinetics in both directions even after facilitation by insulin or anoxia (2, 10, 29). This constellation of properties is difficult to reconcile with the formation and transfer of fluid-containing vesicles across the sarcolemma. Furthermore, the properties of transport are not accounted for most simply by the concept that sugars attach to membrane sites and are carried into or out of the cell by membrane flow as Bennett (30) has postulated in an attempt

to extend the original concepts of Lewis (31) regarding pinocytosis and active transport systems. It is well established in the case of the heart, for example, that glucose transport in the presence or absence of insulin occurs in media containing substantial concentrations of sorbitol without the latter substance appearing in detectable amounts in the intracellular water (11). One would have to suppose that glucose was attached to sites on the cell membrane within invaginations of the plasma membrane and that extrusion of the water containing sorbitol (and other extracellular substances) occurred prior to pinching off of the vesicles to form intracellular inclusions. The electron microscopic pictures, however, show that intracellular vesicles apparently contain substantial quantities of fluid which on dissolution would discharge sorbitol (and other extracellular elements) into the cytoplasm. In an analogous manner, the rapid outward transport of monosaccharides would involve the intracellular formation of vesicles containing the sugar and fluid from which the common intracellular small molecules had been excluded. We would suggest, therefore, that pinocytosis is not the mechanism for glucose transport in the heart under any conditions. It would seem, in fact, that pinocytosis offers a poor alternative to the current concepts of membrane carrier transport (see 32 for review) as a mechanism for transport of rapidly penetrating small molecules. In certain cell types, such as the ameba, increased permeability to small molecules such as glucose may be accomplished by pinocytosis (33) or may simply be the unavoidable consequence of imbibition of extracellular fluid in a process designed for other purposes, such as the relatively slow transport of large molecules or particulate material.

It would seem probable that the effects of insulin in heart muscle and the fat pad stem from the same primary action of the hormone. This primary action could be inferred to lead to pinocytosis in the fat pad and not in the muscle because of differences in the constitution of the tissues and their membranes; *e.g.*, adipose tissue has little or no endoplasmic reticulum, whereas in muscle this organelle is highly developed. We would suggest, however, that pinocytosis is not requisite to accelerated glucose transport. Studies in the ameba (34) indicate that a wide variety of conditions and agents may induce pinocytosis. Although Barrnett and Ball observed insulin-induced pinocytosis in the absence of glucose, it is possible that pinocytosis arose secondary to changes in the permeability to other substances, *e.g.*, inorganic ions. This interpretation would be in accord with one of Barrnett and Ball's suggestions, that insulin-induced pinocytosis in the fat pad is incidental to and not the mechanism for more rapid transport. It has also been suggested that insulin alters membrane permeability by attaching to a membrane sulfhydryl group instigating a chain of thiol-disulfide interchange reactions (35). This mechanism of hormone action could be expected to result in structural alterations in the membrane, but of a magnitude undetectable with the resolution obtained in this study.

The lipid inclusions observed in the diabetic hearts were found in organs sectioned immediately following removal from the animal (Fig. 16). The plasma levels of lipids are increased in diabetic rats (36) and greater uptake of lipid might explain the excessive deposits in the heart. Neither the appearance nor the number of these inclusions was affected by insulin in the period of these experiments. The relationship of these lipid deposits to carbohydrate metabolism in diabetic muscle will be discussed in a subsequent paper.

The lipid inclusions found in normal hearts perfused under anaerobic conditions were not present at the beginning of perfusion. Since the perfusate was devoid of lipid, these inclusions must have originated from lipid already present in the myocardium. The nature of the change in tissue lipid is unknown, but it is possible that the formation of these inclusions is related to the appearance of intracellular particulate fat, which has been observed by light microscopy in the heart in a variety of toxic conditions. The occasional limiting membrane-like structures which were seen may have represented myelin figure forms of phospholipid.

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