

THE ACTION OF INSULIN ON CELLS AND PROTOPLASM ¹

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In a lecture he gave in 1947, Best (1948) stated, "We often hear the statement made that we have had insulin for 25 years and still do not know exactly how it acts. This is quite true, but we know more about the action of insulin than about any other hormone."

Strangely enough, the great science of endocrinology, with its vast body of information concerning the chemistry and the ultimate effects of various hormones, has not been able to solve the basic problem of why the different hormones act as they do. In recent years in the attempt to understand the action of insulin, there has been more and more emphasis on studies of the cell as a whole rather than on studies of fragments or extracts of cells. In a thoughtful review of the literature, Ross (1956) is led to comment (p. 364), "It is apparent that no consistent effect of insulin has been demonstrated in cell-free systems." And the biochemist Levine, a leading authority in the field of carbohydrate metabolism and long a student of insulin, wrote recently (Levine and Goldstein, 1955) (p. 344), "It would be expected therefore that a certain degree of morphological intactness is necessary to demonstrate hormonal effects and actions. Otherwise we would be pulling the trigger of an unassembled gun." Levine himself has turned to the ways of thought and the methods of cell physiology in order to obtain a solution of the insulin problem.

Thus it may not be too presumptuous for cell physiologists to express ideas about the mechanism of insulin action. Indeed back in 1914, Höber suggested that diabetes might be due to a change in the permeability of cells for sugar. This idea was then taken up by Wiechmann (1924, 1926), and by Häusler and Loewi (1925; see also Loewi, 1927). However, the older evidence in favor of the permeability theory was not very convincing, and interpretations in terms of the cell as a whole were pushed into the background by chemical work which seemed to show that insulin had a specific effect on certain isolated enzyme systems. More recently, and on the basis of newer evidence, the permeability theory has been revived and modernized, and it is finding wide support.

No attempt will be made here to review the enormous mass of literature on insulin. Books on endocrinology contain a great deal of information and there have been a number of recent reviews by authorities in the field (Haugaard and Marsh, 1953; Stadie, 1954; Levine and Goldstein, 1955; Weil-Malherbe, 1955; Ross, 1956; Stich and Maske, 1956).

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A few facts stand out. Some of these have been long known and were mentioned in a review published by Macleod in 1924. For our purposes, it may be well to remind the reader that:

1. The action of insulin in reducing the concentration of sugar in the blood is not due to an action on the blood itself, for insulin does not have this effect on blood withdrawn from the body. Hence the cells and the protoplasm they contain must play a part in the lowering of sugar concentration in the blood.

2. The convulsive action caused by excess insulin is not due to an effect on the cerebrum, for it occurs in decerebrate animals.

3. Lowering of the sugar concentration in the medium surrounding isolated nerve or muscle has no effect in stimulating either nerve or muscle.

4. When a rabbit is given a lethal dose of insulin, violent convulsions occur; these are then followed by a comatose stage, and convulsions and coma continue in alternate phases until, after an hour or more, the animal dies.

5. The harmful results of excess insulin can be relieved by the injection into the blood stream of a small amount of glucose.

6. Lack of insulin causes a failure of fat, carbohydrate and protein formation. Best (1953) sums up the situation (p. 434) by stating that "insulin is a central anabolic hormone without which many of the building processes . . . cannot proceed at the physiologic rate."

7. Insulin also causes an increase in the rate of oxidation of sugar. Thus it is a catabolic hormone as well as an anabolic hormone.

It is clear that insulin markedly increases the rate of activity of various enzymic actions, and yet when purified preparations of these enzymes have been tested, there seems to be little or no effect of insulin upon them. Moreover it would be hard to explain how it would be possible for a single substance to have a direct effect on all the various enzymes responsible for the synthesis of carbohydrates, proteins and fats, as well as those responsible for the oxidation of sugar. Hence we apparently must conclude that in one way or another some change in the cell or its protoplasm has an accelerating effect on many types of enzyme activity.

At the present time, what is doubtless the leading theory of insulin action holds that the primary effect of insulin is to change the cell in such a way as to facilitate the passage into it of various sugars. This theory, due in its present form to Levine and his collaborators, has been supported not only by the work of Levine and his group, but also by the careful and ingenious experiments of various other investigators. Perhaps the most impressive work is that of Park, Bornstein and Post (1955). The entire subject is ably reviewed by Ross (1956), and this review should be consulted by anyone interested in details or references to the rather extensive literature. The permeability theory has been enthusiastically endorsed by Stadie (1957), who is certainly one of the outstanding investigators in the field.

Scarcely anyone has attempted to criticize the permeability theory, although such criticism is possible, both from the standpoint of our knowledge of permeability and transport mechanisms, and also because the theory can scarcely offer an explanation of some of the basic known facts of insulin action. This latter point will be discussed in a later section.

EFFECT OF INSULIN ON THE PERMEABILITY TO GLUCOSE

Our experiments with insulin were done entirely on relatively simple, isolated cells of lower organisms. These cells offer exceptionally favorable material for the cell physiologist, but work with such cells is open to the criticism that the action of insulin may be confined to the cells of vertebrate animals. Such an opinion is held by Ross in the review cited above, and it was expressed forcibly by Best, Jephcott and Scott (1932). However, there are reports of insulin action on the cells or tissues of protozoa, sponges, flatworms, crabs and insects; on yeast cells, and on various kinds of bacteria. Whether this literature is sound or not is a question we do not care to discuss. Certainly there can be no objection to our using simple living cells to explain insulin action, for if we can obtain effects on these cells with insulin and if such effects can be used to interpret the action of insulin in higher animals, we may be able to offer suggestions of some value.

For the study of cells and protoplasm the eggs of marine invertebrates offer many advantages and this type of material has often been used by cell physiologists. We used the eggs of the sea urchin *Arbacia punctulata*, the surf clam *Spisula solidissima*, and the annelid worm *Chaetopterus pergamentaceus*. All of these eggs are readily available at Woods Hole. If insulin directly favors the entrance of glucose into cells (quite apart from any indirect effect it might have as a result of the utilization or combination of glucose within the cell), we thought that perhaps we might be able to find evidence for such a direct effect on marine egg material. This we were unable to do, and our results were wholly negative. For this reason we shall not attempt to report them in any detail, but will merely cite a few of our experiments as briefly as possible.

One of the standard ways of determining the ease with which dissolved substances pass through cell membranes is to study the osmotic behavior of cells in relation to solutions of the substances in question. There are a variety of such osmotic methods. One of the simplest of these methods depends on the fact that the more readily a substance penetrates, the less osmotic pressure it can exert against the plasma membrane of the cell. By observing the changes in volume of the cells when they are immersed in various concentrations of a given substance, one can obtain a rapid measure of the ease with which a substance enters. In our particular case, if insulin favored the entrance of glucose, a solution of glucose containing insulin would be less potent osmotically than a similar control solution which differed only in lacking insulin. In order to obtain as great an effect as possible, we used saturated solutions of insulin. These were obtained by dissolving 0.2 mg. of insulin in one ml. of the glucose solution. Actually not all of the insulin went into solution. All the solutions were brought to the pH of sea water. The insulin we used was a preparation which was relatively zinc-free; it was kindly supplied by the Eli Lilly Company through the kindness and courtesy of Dr. G. H. A. Clowes. In view of the fact that many types of protoplasm are very sensitive to zinc, we were indeed fortunate to obtain this preparation from which 98.8% of the zinc ordinarily present in crystalline insulin had been removed. Actually an assay made by the Eli Lilly Company showed only 0.0061% zinc in the dry material.

Table I shows the results of our experiments on sea urchin eggs. In this table, as also in Tables II and III, the \pm sign indicates standard deviation. A molar solution of glucose caused a slight decrease in the volume of the eggs. In the

TABLE I

Effect of glucose and glucose + insulin on the osmotic behavior of Arbacia eggs. Measurements of diameters were made after the eggs had been immersed in the solutions for 5 minutes. The values show the average of 10 measurements

Diameter of eggs in sea water	77.8 \pm 2.02 microns
Diameter of eggs in molar glucose solution	71.5 \pm 2.18 microns
Diameter of eggs in molar glucose solution containing insulin	70.7 \pm 2.29 microns

presence of insulin approximately the same decrease occurred. If insulin had favored the entrance of the glucose, then the solution of glucose containing the insulin should not have caused as great a shrinkage.

Similar results were obtained with eggs of the clam *Spisula*, as is shown in Table II. Again there is no indication that insulin favors the entrance of glucose.

In our experiments with eggs of the worm *Chaetopterus*, we ran into difficulty. When we immersed these eggs in solutions of glucose, the sugar entered rapidly, so rapidly in fact that even solutions as strong as 2 *M* caused no shrinkage of the eggs. We experimented with 2 *M* and 1.75 *M* and 1.5 *M* glucose solutions with and without insulin. In all cases, in the absence of insulin the glucose entered more rapidly than when it was present. In the 1.75 *M* and 1.5 *M* glucose, frequently the eggs swelled so rapidly that they broke. This sometimes made measurements uncertain. Apparently in the glucose solutions, absence of ions like calcium changed the semipermeable membrane of the cell in such a way that it became permeable to glucose. Perhaps the small amount of zinc in the insulin helped partially to stabilize the membrane. Because of the increased permeability of the cell membrane in the absence of the salts of sea water, we decided to compare the behavior of glucose and glucose + insulin in the presence of an appreciable amount of sea water. The results are shown in Table III. In this case also, presence of insulin does not favor the entrance of glucose.

Our results with marine eggs lead to the conclusion that the effect of insulin in increasing the rate of entrance of glucose into cells is not a general phenomenon true for all types of living material. Other authors in the past have reached the same conclusion. Thus it is now commonly held that insulin does not increase the rate of entrance of glucose into the erythrocytes of man and mammals. However, the literature on erythrocytes is, or at least has been, highly controversial (see Foshay, 1925; Häusler and Loewi, 1925; Loewi, 1927; Höglér, Thomann and Überrack, 1929; Himmerich and Tschernjak, 1936; also many papers cited by them; Wilbrandt, 1947; Guensberg, 1947; Pletscher, von Planta and Hunzinger,

TABLE II

Effect of solutions of glucose and glucose + insulin on the osmotic behavior of Spisula eggs. Measurements of diameters were made after the eggs had been immersed in the solutions for 5 minutes. The values are in microns; they show the average of 10 measurements

	0.9 <i>M</i>	0.8 <i>M</i>	0.7 <i>M</i>
Diameter of eggs in sea water	61.0 \pm 2.28	56.8 \pm 1.67	56.4 \pm 1.07
Diameter of eggs in glucose	56.8 \pm 1.78	56.4 \pm 1.52	58.9 \pm 0.83
Diameter of eggs in glucose + insulin	53.7 \pm 1.77	53.5 \pm 3.80	54.1 \pm 2.87

TABLE III

Effect of glucose and glucose + insulin on the osmotic behavior of Chaetopterus eggs.

Solution A = 4 parts molar glucose solution + 1 part sea water.

*Solution B = 4 parts molar glucose solution containing
insulin + 1 part sea water*

Control eggs in sea water measure 99.4 ± 2.06 microns

Eggs in A (glucose alone) measure		Eggs in B (glucose + insulin) measure	
After 3 minutes	94.0 ± 1.63 microns	After 3 minutes	94.2 ± 1.83 microns
After 136 minutes	95.4 ± 2.50 microns	After 135 minutes	94.0 ± 2.58 microns

1955). Any interpretation of this literature is complicated by the fact that glycolysis and other changes in carbohydrates may well occur in blood cells. Park and Johnson (1955) failed to find any increase in the rate of entrance of glucose and galactose into rat brain cells when insulin was present, but here, too, the results are based on the assumption that under the conditions of the experiment both glucose and galactose remained unaltered when they entered the cells, and this conclusion may not be entirely warranted (compare Sols and Crane, 1954).

If, as is now commonly believed, the favorable effect of insulin on the transport of sugar into cells is due to some sort of an enzyme-controlled reaction, then acceleration of this transport promoting enzyme might well be the cause of the increase in the rate of entrance of sugar. Thus the more rapid transport of sugar into cells in the presence of insulin might merely represent one aspect of the general effect of insulin in accelerating diverse types of enzyme activity. In other words, the more rapid entrance of sugar, instead of being the basic reason for insulin action, might be a result rather than a cause of some underlying change that is responsible for a general increase in enzymic activity. What could such a cause be?

EFFECT OF INSULIN ON COLLOIDAL CHANGES IN PROTOPLASM

When various types of cells are excited by stimuli of one sort or another, calcium is released from the cell cortex, and this calcium then activates a proteolytic enzyme system. The proteolytic enzyme also serves as a clotting enzyme and produces a gelation of protoplasm in the interior of the cell. This gelation involves an oxidation of $-SH$ to $S-S$ groups. Thus the release of calcium can result in an increase in protease activity and also an increase in cellular oxidations. In other words, calcium release is the trigger that starts off a number of enzymic reactions. The evidence on which these statements is based has been presented in considerable detail in recent books (Heilbrunn, 1956, 1958); see also Wilson and Heilbrunn, 1957.

Could it be possible that in one way or another insulin might act in somewhat the same way that stimulating agents do, and what could conceivably be the reason for such an action? This possibility is what intrigued us and induced us to undertake the work that is described below.

If gelation and the reactions underlying gelation in protoplasm constitute the trigger for protoplasmic activity, then presumably protoplasm must have some

method of braking or inhibiting the gelation. In the books just referred to, strong evidence is presented to show that heparin and heparin-like substances can constitute such a brake. Heparin not only can inhibit protoplasmic gelation in much the same way that it inhibits blood clotting, it can also inhibit the action of various types of enzymes. In living cells generally, there seems to be a balance between the factors which tend to induce gelation or clotting and those which tend to prevent it. Heparin (and/or heparin-like substances) is one of the inhibiting factors. If we could imagine a substance which would antagonize or neutralize the effect of heparin and similar substances, then it might well act to accelerate various enzyme systems in the protoplasm. Insulin is such a substance, as we shall now attempt to show.

Sol-gel reactions undoubtedly occur in many, if not all types of protoplasm, but they are especially evident in the ameba. Moreover, in the ameba, a small amount of heparin can be shown to prevent the clotting reaction which normally occurs whenever the cell is torn or broken, that is to say the surface precipitation reaction. We use the giant ameba, *Chaos chaos*, and the heparin we used in our experiments was kindly supplied by the Upjohn Company. If an ameba is immersed in a dilute solution of heparin, say a 0.01% solution, and the ameba is crushed by exerting pressure on the coverslip over the animal, no surface precipitation reaction occurs and the contents of the ameba flow out through the solution. However, if the heparin solution is also made to contain a 0.01% solution of insulin, there is an excellent surface precipitation reaction and the exuding protoplasm forms a distinct membrane about itself.

We performed a series of experiments in which various concentrations of insulin were balanced against various concentrations of heparin. In deciding whether or not a surface precipitation reaction occurs, it is important not to vary too greatly the amount of pressure with which the ameba is broken. For with too great pressure and with too rapid emergence of the interior protoplasm, there is scarcely time for a proper reaction to occur. It is difficult to measure the amount of pressure applied to a coverslip. In order to measure this pressure, one of us (Ashton) devised an apparatus in which a small rectangular piece of glass was attached to a lever which in turn was attached to a DeNouy tensiometer. With this apparatus it is possible to measure the amount of pressure applied before a cell breaks. The measurements are not very exact, but they have the advantage of being objective. For cells which do not vary greatly in volume, as for example sea urchin eggs, the pressure required to break them, as indicated by our apparatus, is reasonably constant. However, for amebae which differ markedly in size, as do our specimens of *Chaos chaos*, the breaking pressure varies more widely, for with the larger amebae there is more resistance to the pressure imposed upon them. Table IV shows what happens when amebae are broken in mixtures of insulin and heparin. The last column indicates whether or not a surface precipitation reaction occurred and whether it was a strong or a weak reaction. The amount of pressure required to break the amebae is also recorded. As was to be expected, this pressure varied widely, but whether the pressure was relatively great or relatively small, the results were always the same. It is clear, therefore, that the effect of heparin in preventing the protoplasmic clotting necessary for the surface precipitation reaction can be completely blocked by the addition of insulin. Moreover, a control test showed

that the amount of zinc present in our solutions had no such effect. It should be noted that a given amount of insulin can neutralize four times as much heparin.

Further evidence of a combination between insulin and heparin is provided by experiments in which it was shown that the metachromatic reaction of heparin with toluidine blue was prevented by solutions of insulin. In these experiments, shown in Table V, relatively large amounts of insulin were necessary to block completely the metachromatic reaction. Here we are dealing with a system in which only insulin and heparin are present, whereas in the earlier experiments the system included not only insulin and heparin, but also the protoplasm of the ameba. Probably the protoplasm, or rather some proteins contained in it, have an affinity for heparin and can unite with it in spite of the presence of insulin. At any rate, this might constitute an explanation of the different types of ratios obtained in the two experiments. Another explanation might be that it may take more insulin to block

TABLE IV

The effect of mixtures of heparin and insulin on the surface precipitation reaction of Chaos chaos

% insulin	% heparin	Ratio insulin/heparin	Pressure in milligrams	Spr
0.01	0.01	1-1	120	strong
		1-1	88	strong
		1-1	92	strong
		1-1	120	strong
		1-1	160	strong
0.01	0.02	1-2	208?	strong
		1-2	140	strong
		1-2	116	strong
		1-2	52	strong
		1-2	96	strong
0.01	0.03	1-3	116	weak
		1-3	124	strong
		1-3	188	weak
		1-3	48	weak
		1-3	116	weak
0.01	0.04	1-4	48	weak
		1-4	168	weak
		1-4	128	weak
		1-4	252?	very weak
		1-4	76	very weak
0.01	0.05	1-5	96	none
		1-5	60	none
		1-5	134	none
		1-5	68	none
$2 \times 10^{-7} M$ zinc	0.01		124	none
			125	none
			144	none
			172	none
			145	none

TABLE V

Metachromatic reaction of mixtures of heparin and insulin. One milliliter of a 0.02% insulin solution was mixed with an equal volume of various concentrations of heparin, and the various mixtures were then tested for metachromasia with 6 drops of a 0.01% solution of toluidine blue

% insulin	% heparin	Ratio insulin/heparin	Reaction
0.02	0.02	1	+
0.02	0.01	2	+
0.02	0.0067	3	+
0.02	0.005	4	+
0.02	0.004	5	+
0.02	0.0033	6	+
0.02	0.00286	7	+
0.02	0.0025	8	+
0.02	0.0022	9	+
0.02	0.002	10	+
0.02	0.00182	11	+
0.02	0.00167	12	+
0.02	0.00154	13	—
0.02	0.00142	14	—
0.02	0.00134	15	—

the metachromatic reaction of heparin than it does to block its effect on clotting or on enzymic action.

When amebae are stained with toluidine blue, the outer region of the cell gives a beautiful metachromatic color, a color such as that which would be given by heparin or a heparin-like substance. But if amebae are immersed in solutions of insulin for some hours, staining with toluidine blue no longer gives a metachromatic reaction. Such a loss of the metachromatic reaction occurs even in very dilute solutions of insulin. This is shown in Table VI. In interpreting this table, it should be remembered that the amebae were immersed in solutions whose volume was very large in comparison with the volume of the amebae. Actually in the experiments reported in the table, 10 ml. of solution were used and only a few drops of a concentrated suspension of amebae.

Our experiments indicate that insulin can and does combine with heparin. There is some indication in the chemical literature in support of this view. According to Gorter (1954), heparin can combine with various proteins, including insulin. In Gorter's experiment, the insulin was combined with the lipid cephalin

TABLE VI

Metachromatic reaction of Chaos chaos after the amebae were immersed for 16 hours in various concentrations of insulin solution

Concentration of insulin, %	Reaction
0	+
0.000625	+
0.00125	—
0.0025	—
0.005	—

(phosphatidyl-serine), and Gorter believes that as a result of the complex formed between insulin and heparin, the lipid is set free. The reaction between heparin and insulin is strongly influenced by hydrogen ion concentration, a fact which may be of considerable importance in the interpretation of biological phenomena.

DISCUSSION

Although the permeability theory of insulin action has been so widely accepted, as already noted, the fact that insulin increases the rate of passage of sugar into a cell could well be the result of some acceleration of an enzyme responsible for such transport, so that the more rapid entrance of the sugar would really be a result rather than a cause of enzyme action. A somewhat similar idea was expressed many years ago by Staub (1927). But this type of objection is perhaps not too serious. The value of a theory lies in the extent to which it can explain and interpret known facts. Perhaps the most important fact about the action of insulin is that it behaves as an anabolic hormone and produces an increase in the synthesis of carbohydrates, proteins and fats. It could of course be claimed that inasmuch as insulin hastens the entrance of sugar into a cell, this fact in itself might favor the synthesis of proteins, for the energy for such syntheses is now believed to come from the oxidation of carbohydrate. This may well be the correct explanation. However, according to Sinex, MacMullen and Hastings (1952), the addition of glucose tends to prevent the insulin-induced synthesis of protein in the rat diaphragm. And if we consider the long-known facts concerning the effects of insulin, the permeability theory would have some difficulty in offering a complete explanation. For if the primary effect of the hormone is to increase the sugar content of a cell, then logically one should be able to imitate the effects of insulin merely by feeding or injecting excess glucose, for such glucose would also increase the cellular sugar. But administration of excess sugar is hardly a cure for diabetes. Moreover, under conditions in which there is an excess of insulin in the blood, if the sugar permeability theory were correct, from a logical standpoint the very worst possible treatment to offset the harmful effects of the excess would be to feed or inject glucose; for the permeability theory assumes that insulin acts by introducing more glucose into the cells. And yet, as is standard knowledge, the convulsions and other adverse symptoms induced by excess insulin can be cured readily enough by the administration of glucose.

Of course in an animal as complicated as a mammal, there are too many interactions of various organ systems to enable one to reach unassailable conclusions by logic based on the behavior of any one organ system. It is quite possible that insulin and sugar have one effect on muscle and another on the cells of the medulla and spinal cord. Thus it might be postulated that in insulin shock, the nerve cells in the basal part of the brain come to lack glucose and that this lack is responsible for the convulsions and the coma. Then the administration of sugar might quickly restore the nerve cells to their normal state.

On the basis of our theory, if insulin acts primarily by combining with heparin and counteracting its effects, then such an action would immediately accelerate various types of enzymic activity. For, as is well known, heparin is an inhibitor of some proteases. Thus, it inhibits the action of trypsin (Horwitt, 1940), and pepsin (Marini and Levey, 1955). It also inhibits the action of ribonuclease

(Zöllner and Fellig, 1953), and of amylase (Myrbäck and Persson, 1952). Because it is a polyanion, it can have a retarding effect on the activity of various enzymes (Spensley and Rogers, 1954). When injected into the blood stream, it tends to extract lipases from tissues (Iselin and Schuler, 1957), and presumably this would retard cellular lipase activity. And inasmuch as it retards the activity of ribonuclease, it might also tend to prevent the synthesis of ribonucleoproteins; this might be a factor in retarding the formation of proteins with enzymic activity. Finally, because heparin prevents the clotting of protoplasm, and such clotting, as stated previously, acts as a trigger for oxidative reactions, it might also retard the oxidative activity of a cell. Hence by the simple combination with heparin, insulin could exert many of the effects we know it to have. In support of our point of view, it might be noted that according to Bond and Spitzer (1955), much of the hypoglycemic effect of insulin is lost if it is injected into rabbits previously injected with heparin. Bond and Spitzer do not believe that this phenomenon is due to any combination of heparin with insulin, for when they injected a mixture of the two substances, insulin action remained unimpaired. But within blood, and even more within cells, various factors such as pH, ionic strength or even the presence of protein co-factors, might have an influence on any possible combination.

As recent authors are coming to realize (Weissbecker and Hitzelberger, 1953; Riley, Shepherd, West and Stroud, 1955), heparin has many physiological actions in addition to its effect on blood clotting. Thus Riley *et al.* suggest "that the function of heparin may be concerned rather with events in the tissues than with the coagulability of the circulating blood," and a similar statement is also made by Weissbecker and Hitzelberger. One interesting phenomenon is the fact that heparin antagonizes the effects of ACTH and cortisone. As we learn more about the heparin and heparin-like substances that are found in cells, we may gain additional insight into life processes and the action of various drugs on these processes.

Obviously the work we have done represents only a beginning. If the theory we propose is correct, then much more work needs to be done in order to place it on a firm footing. Any theory which attempts to give a complete explanation of insulin activity is faced with many difficulties.

SUMMARY

1. Insulin does not speed the entrance of glucose into the eggs of a sea urchin, a clam and a worm.
2. Dilute solutions of heparin prevent protoplasmic clotting in ameba. This action of heparin is blocked by insulin.
3. Evidence is presented to show that insulin combines with heparin. It blocks the metachromatic reaction that heparin gives with toluidine blue. This can clearly be shown *in vitro*, and it is also indicated by studies on living amebae.
4. Earlier work has shown that heparin acts as an inhibitor of various enzymes, and in general it may be thought of as constituting a brake on many of the chemical activities of a cell. By preventing this inhibiting action, insulin is able to promote the synthesis of various essential constituents of the protoplasm.
5. Also, in view of the fact that protoplasmic clotting involves oxidation and can act as a trigger for oxidative activity, insulin by preventing the anticlotting action of heparin can promote oxidations.

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