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Alleviation of IgE-mediated immune reactions in hypoinsulinaemic and hyperglycaemic mice

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SUMMARY

Alloxan diabetic mice do not easily develop anaphylactic shock, and formation of IgE antibodies to sensitizing antigen almost ceases in these animals. Also, mice sensitized as normoglycaemic and then made diabetic are to a great degree protected from anaphylaxis, although they form minute but measurable amounts of IgE antibodies. Immune cells transferred into diabetic mice lose their ability to form IgE, while cells from immune diabetic mice, which in the hypoinsulinaemic milieu of donors do not synthesize appreciable amounts of IgE, regain this ability upon transfer into normal recipients. We conclude that the IgE response is very insulin-dependent and that insulin deficiency affects IgE-forming cells directly or indirectly via its influence on T helper cells. In the current study we also considered whether hyperglycaemia may influence the effector stage of anaphylactic reactions. To test this, massive doses of glucose were given to normoglycaemic mice, which increased their blood glucose level to that seen in diabetic mice and prevented local anaphylactic reactions when these mice were injected with monoclonal IgE antibody and challenged locally with antigen.

INTRODUCTION

Hypoinsulinaemia (hyperglycaemia) severely impairs cell-mediated immune responses (CMI). Abrogation of contact sensitivity reactions, and decrease of PHA or MLC responses *in vitro* were found in alloxan-induced diabetes and/or in pancreatectomized animals (Ptak, Czarnik & Hanczakowska, 1975; Fabris & Piantanelli, 1977). The effect of hypoinsulinaemia on antibody production is less clear (Dolkart, Halpern & Perlman, 1971; Fabris & Piantanelli, 1977), but in many experiments (Ptak, Hanczakowska & Rozycka, 1977), as well as in clinical patients (Moen & Reiman, 1933; Bates & Weiss, 1941), a decrease of antibody responses as well as in CMI have been found.

There is evidence suggesting that insulin may exert a significant influence on anaphylactic reactions (Swern, 1932; Abrahamson, 1941; Sanyal, Spencer & West, 1959; Thompson, 1961; Ganley, 1962; Adamkiewicz, 1963; Ottlecz *et al.*, 1978). Insulin administration increased the mortality from anaphylactic shock in sensitized mice and rats (Sanyal *et al.*, 1959; Thompson, 1961; Ottlecz *et al.*, 1978). In contrast, alloxan diabetes had a protective effect which could be reversed by insulin (Thompson, 1961; Ganley, 1962). A similar protection was observed in hyperglycaemia induced by a massive dose of glucose given before the eliciting dose of antigen (Thompson, 1961). The question can thus be posed whether the resistance of diabetic subjects to anaphylactic reactions

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is due to deficiency of insulin or to an excess of glucose, since the mechanisms of protection in either of these cases would be quite different.

Experiments reported here were intended to test whether hypoinsulinaemia and/or hyperglycaemia function at the afferent or effector phase of anaphylactic reactions. We also wanted to know whether hypoinsulinaemia impairs the production of reaginic antibodies, prevents degranulation of mast cells or only makes the animals refractory to the action of mediators released during immune reactions.

MATERIALS AND METHODS

Animals. Male CBA mice, 10–12 weeks old from our own mouse colony were used throughout. Lewis male rats were purchased from the local supplier.

Reagents. The following reagents were used: picryl chloride (Fluka A.G., Buchs, Switzerland); normal horse serum and pertussis vaccine (Biomed, Cracow, Poland); alloxan (International Enzymes Ltd, Windsor, UK); Evans blue (Matheson, Coleman & Bell Inc., Norwood, Ohio); cyclophosphamide (VEB, Jenapharm, DGR); insulin semilente (Polfa, Warsaw, Poland); IgE–hapten affinity purified murine monoclonal DNP/TNP-specific IgE antibody (Liu *et al.*, 1980) was a gift of Dr Philip Askenase, Yale University.

Alloxan diabetes and hyperglycaemia. Mice were injected with alloxan (75 mg/kg) on two occasions, 4 days apart. Blood glucose level tested 7 days after the second injection was in the range 180–750 mg/dl. Unless otherwise stated only mice with blood glucose level exceeding 280 mg/dl were used. Hyperglycaemia was produced in mice by i.p. injection of 250 mg glucose in 2 ml saline 30 min before the eliciting dose of antigen. Since in these animals blood glucose levels returned to almost normal values after 4 h, in some experiments mice received a second injection of 150 mg glucose (in 2 ml) 2 h later. At the time of antigen administration the blood glucose level in these animals was in the range 350–470 mg/dl, and declined after 4 h to 200–250 mg/dl.

Sensitization and elicitation of anaphylactic shock. The procedure we used was similar to that used by Malkiel & Hargis (1952). For sensitization mice recived i.p. 0·05 ml horse serum diluted 1:4 and 4×10^9 *Bordetella pertussis* vaccine. Ten days later, unless otherwise stated, mice were challenged i.v. with 0·1 ml horse serum. Anaphylactic deaths were recorded after 4 h. Diabetic mice were sensitized 7 days after the second injection of alloxan. In some experiments mice sensitized 9 days before underwent diabetogenic regime, as described above. The anaphylactic shock was elicted 3 days after the second dose of alloxan.

Local IgE-mediated reaction. This was done as described by Askenase, Rosenstein & Ptak (1982). Briefly, normal animals, or animals that were injected i.p. with glucose 30 min previously, were injected i.v. with 30 μg of TNP specific IgE in 0·5 saline and both sides of their ears were challenged by topical application of 0·8% picryl chloride in olive oil. Increase of ear thickness was measured with an engineers micrometer at different time intervals, and increment was expressed as the mean $\pm$ s.d. in units of 10^{-3} cm (Asherson & Ptak, 1968). Non-injected controls were challenged and measured similarily.

Passive cutaneous anaphylaxis. The flanks of rats were shaved with electric clippers. On the next day they were injected intradermally with 0·05 ml of mouse immune serum diluted 1:3, 1:9, and 1:27. For challenge rats kept at 22°C were injected 24 h later into the tail vein with antigen (0·1 ml) in 0·5% Evans blue dye solution (total volume 0·5 ml). Thirty minutes later rats were killed, skin opened and the diameters of blue spots on the under surface were measured. Results are expressed as the mean $\pm$ s.d. of average diameter. Results with mouse sera diluted 1:3 are only shown because when decreasing the amount of serum the reactions became smaller, but gave similar results.

Transfer of cells. Single cell suspensions were prepared from spleens and lymph nodes of normal or diabetic mice that were sensitized to horse serum 10 days previously. These cells (5×10^7) were injected i.v. into normoglycaemic or diabetic mice treated with cyclophosphamide (180 mg/kg) one day before transfer. Recipient mice were immunized immediately with horse serum and *B. pertussis* and challenged with antigen 10 days later. At the day of challenge the blood glucose level in diabetic recipients varied between 280 and 470 mg/dl.

Treatment with insulin. Thirty minutes before the eliciting dose of antigen mice were injected s.c. with 1·5 iu of insulin. This dose at the time of antigen administration decreased the blood glucose level in experimental animals to 80–200 mg/dl, and to 30–60 mg/dl in control mice.

Estimation of blood glucose level. This was done by the *o*-orthotoluidine method (Hyvarinen & Nikkila, 1962).

RESULTS

Anaphylactic shock in normoglycaemic and diabetic mice (Table 1)

In eight independent experiments the mortality rate in normoglycaemic mice varied between 60 and 100% (mean = 79%). Although anaphylactic shock was elicited routinely on day 10 after sensitization, an identical mortality rate was observed in mice challenged on day 16 (control group to animals first sensitized and than made diabetic). In mice sensitized after development of diabetes the corresponding values were 0–22% (mean 13%). The few diabetic mice that developed fatal shock had blood glucose levels indistinguishable from survivors and their sera contained very low levels of IgE as estimated by PCA reaction (see below). In two experiments we also observed that even moderate hypoinsulinaemia (glucose level 180–230 mg/dl) may significantly protect animals against development of fatal shock (33% mortality). Almost identical results were observed when diabetes was induced in already sensitized animals. In these animals hypoinsulinaemia was associated with protection of most mice against anaphylactic shock.

Correlation between the severity of anaphylactic reaction and PCA (Table 2)

While sera of normoglycaemic sensitized mice mediated significant PCA reactions, no reaction, or a very faint one, was seen when sera of mice made diabetic before sensitization were tested. Sera of mice in which diabetes was induced after sensitization elicited some reaction, although significantly lower than positive control sera.

The effect of insulin administration on the anaphylactic shock

Insulin had no effect on the mortality rate either in animals made diabetic prior to sensitization or in normoglycaemic controls but it aggravated anaphylactic shock in mice in which diabetes was induced after sensitization (compare groups C and D) (Table 3).

Table 1. Severity of anaphylactic shock in normoglycaemic and diabetic mice

Treatment of mice (group)	Blood glucose level, mg/dl (range)	Mortality (%)
Alloxan + sensitization (8)*	330–765	41/312 (13)
Alloxan + sensitization (2)	180–230	23/70 (33)
Sensitization + alloxan (4)	370–585	40/165 (24)
Sensitization only (8)†	70–110	304/386 (79)

Mice were sensitized with horse serum before or after development of alloxan diabetes. Control mice were only sensitized. Anaphylactic shock was elicited 10 days (in group C, 16 days) after immunization, and deaths recorded after 4 h. Table shows the combined data from 2 to 8 independent experiments in each group. * Number of experiments. † This group includes animals tested 10 or 16 days after sensitization and the results were combined because identical mortality was noted.

Table 2. PCA reaction in rats injected with mouse immune serum

Treatment of mice (serum donors)	Day serum obtained	PCA reaction in rats mean diameter ± s.d. (in mm) (No. of sera tested)
Alloxan + sensitization	10	0 ± 0 (25)*
Sensitization only	10	15 ± 6 (10)
Sensitization + alloxan	16	5 ± 1 (11)
Sensitization only	16	12 ± 4 (10)

Mice were sensitized to horse serum before or after alloxan administration, or were only sensitized. Rats were injected intradermally with sera of mice and 24 h later received antigen and Evans blue i.v. Thirty minutes later the diameters of blue spots on the inner surface of the skin were measured. Sera were diluted 1 : 3.
* Three mice died of anaphylactic shock.

Table 3. Influence of insulin on the mortality rate from anaphylactic shock

Treatment of mice (group)	Insulin	Blood glucose level, mg/dl (range)	Mortality (%)
Alloxan + sensitization	−	370–520	3/30 (10)
Alloxan + sensitization	+	340–680*	6/34 (18)
Sensitization + alloxan	−	310–490	6/28 (21)
Sensitization + alloxan	+	300–410*	22/32 (68)
Sensitization only	−	70–100	23/28 (84)
Sensitization only	+	70–100*	29/32 (91)

Mice sensitized before or after being made diabetic, and normoglycaemic sensitized controls received before eliciting dose of antigen 1·5 iu of insulin s.c. * Blood glucose level before administration of insulin.

Table 4. Transfer of immediate hypersensitivity by immune cells

Treatment of recipients	Treatment of donors	Mortality of recipients (%)
Cyclophosphamide	Sensitization	14/30 (47)
Cyclophosphamide	Alloxan + sensitization	12/30 (40)
Alloxan + cyclophosphamide	Sensitization	1/33 (3)
Alloxan + cyclophosphamide	Alloxan + sensitization	0/28 (0)

Recipient mice, normoglycaemic or diabetic, were injected with cyclophosphamide (180 mg/kg) and on the next day received spleen and lymph node cells (5×10^7) from normal or diabetic donors sensitized to horse serum. Fourteen days later recipients were challenged i.v. with antigen. The blood glucose level in diabetic donors or recipients was in the range 280–470 mg/dl.

Transfer of immune cells (Table 4)

Immune cells from normoglycaemic or diabetic donors when injected with antigen into normal recipients transferred the ability to develop sensitization. After 14 days, challenge with antigen caused a proportion of these animals to die of anaphylactic shock. In contrast, transfer into diabetic recipients, independent of the cells transferred, was unsuccesful and practically no deaths were recorded in this group.

Influence of glucose administration on systemic anaphylactic shock and a local IgE-mediated skin reaction (Fig. 1)

Injection of glucose into sensitized control mice in doses which significantly increased the blood glucose level had only a marginal effect on the severity of anaphylactic shock. While the mortality rate in control sensitized animals was 75% (24 of 32), 58% mortality rate was noted in mice that received glucose before antigen challenge (19 of 33). In contrast, glucose administration significantly inhibited a local IgE-mediated reaction. While ear swelling reactions in control mice peaked at 1 h and declined thereafter development of reactions in glucose-injected animals had a protracted course, and the magnitude of reactions, which was inversely related to blood glucose level, reached its highest level after 6–8 h. When, however, antigen challenge was postponed until 5 h after glucose injection, control and treated animals showed identical ear swelling reactions. It was concluded that high glucose levels affects the ability to develop an immediate hypersensitivity skin reaction.

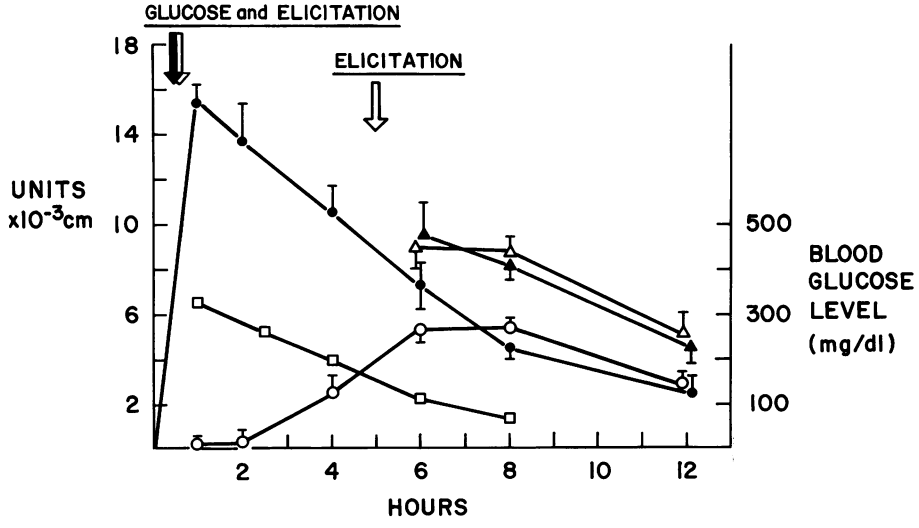

Fig. 1. The time course of local IgE-mediated reaction. Mice treated intraperitoneally (○——○) or not (●——●) with glucose (⇩) were injected i.v. 30 min later with 30 μg of TNP specific IgE monoclonal antibody and immediately challenged on the ears with picryl chloride (⇩). Some mice injected (△——△) or not (▲——▲) with glucose were challenged on ears 5 h later (⇩). The ear increment at each time point is expressed in units 10^{-3} cm. Each point represents the mean ± s.d. of four animals tested (negative control values 2·5 ± 0·74 are subtracted). Blood glucose level in mg/dl (□——□).

DISCUSSION

Normoglycaemic mice can be sensitized easily to horse serum and die of anaphylactic shock when challenged with antigen 10 or 16 days later. This same immunizing procedure does not lead to sensitization in the majority of diabetic mice. Also in animals sensitized before development of diabetes the mortality rate from anaphylactic shock was significantly reduced compared to normal controls. Thus hypoinsulinaemia may prevent sensitization when the antigen is injected into an

already diabetic mouse, or shut down the ongoing synthesis of IgE antibodies when the diabetogenic regime is applied after sensitization. In the latter case since Bε cells are continuously recruited into antibody secreting status and IgE immunoglobulins have an extremely short half life (Peeters & Carter, 1978), the amount of circulating IgE antibodies after several days of diabetic disease decrease to insignificant levels. These findings strongly suggest that IgE responses are highly dependent on the presence of insulin both at afferent and efferent levels, much more than the production of IgM and IgG antibodies (Ptak *et al.*, 1977). Refractoriness of diabetic mice to induction of anaphylaxis could be explained most easily by either the total absence of reaginic antibodies (diabetes and sensitization) or their significantly diminished level (sensitization and diabetes). Since, however, a fatal outcome of anaphylaxis was also noted in some apparently IgE negative diabetic mice, lack or decreased levels of IgE antibodies in the circulation cannot be solely responsible for the protecting effect of hypoinsulinaemia.

The mechanisms by which insulin deficiency influences some immune responses (see Introduction) are not completely clear at present but presumably fall into two, not mutually exclusive categories—direct action on cells responding positively to antigen, and/or indirect influence on these cells throught regulatory (suppressor) cells. Our finding that sensitized diabetic mice do not form IgE antibodies in significant quantities, but their immune cells do so when transferred into normoinsulinaemic environment may be interpreted as indicating that insulin has a direct influence on lymphocyte activity. Although neither resting lymphocytes of B or T lineage have insulin receptors they develop insulin binding sites on their surfaces when stimulated with antigens or lectins (Helderman & Reynolds, 1978). The target of insulin deficiency may thus be the IgE producing cell itself or, since it is known that IgE responses are exclusively T cell-dependent (Michael & Bernstein, 1973), helper T cell, or both. Our data do not clearly support either of these contentions. We cannot, however, rule out completely the presence of suppressor cells in the system we were studying. It has been found previously that diabetic mice when skin sensitized with picryl chloride develop preferentially Ly 2 suppressor cells (Ptak, Rewicka & Kollat, 1980; Ptak *et al.*, 1981). These cells when transferred together with immune Ly 1 cells into normal recipients suppress in short term experiments (24 h) the passive transfer of contact sensitivity but their activity disappears several days after 'parking' in normoglycaemic milieu (Ptak *et al.*, 1980). Our numerous attempts using several experimental procedures to detect suppressor cell activity in anaphylactic diabetic mice failed (data not shown). It is thus likely that even if suppressor cells were present in diabetic mice sensitized to horse serum, experimental conditions we used would make them undetectable.

The action of insulin on anaphylactic reactions is, however, more complex. It has been found that insulin injected shortly before antigen administration in doses that did not influence the blood glucose level, potentiates anaphylactic shock (Ottlecz *et al.*, 1978). This suggests that the target of its action is either mast cells or basophils, the effector cells of anaphylactic reactions or, alternatively, that insulin makes target cells in end organs, such as vessels, more sensitive to the action of mediators that are released during reactions. In our experiments we found that insulin had virtually no influence on the severity of shock in sensitizied diabetic animals, while in sensitized mice made diabetic later it increased somewhat the mortality rate. Since only animals of the latter group have a low level of circulating IgE insufficient *per se* to produce fatal anaphylaxis in the majority of mice, insulin administration clearly enhanced either the release of mediators or sensitivity to their action.

Although these effects could be due to the direct action of insulin on target cells it is also likely that high glucose concentration might affect perhaps by increased osmotic pressure the outcome of antigen–antibody reactions on the surface of mast cells or the overall reactivity of other cells to the action of mediators. Thus insulin may influence the response indirectly by decreasing the blood glucose level. In our experiments glucose injection had only marginal effect on the outcome of anaphylactic shock despite the fact that its concentration rose after injection to the level recorded in diabetic animals which had no signs of anaphylactic reactions. The interpretation of this finding is difficult because animals in both groups, diabetic and hyperglycaemic, differed in the level of circulating antibodies. Clear-cut evidence, however, that high glucose level can significantly affect the effector stage of immediate hypersensitivity was demonstrated in the local IgE-mediated reaction. Here, as long as blood glucose levels remained high there were no signs of local skin erythema and swelling.

Our experiments demonstrate that the mechanism of action of insulin on anaphylactic phenomena is complex. Insulin depletion may prevent triggering of IgE producing cells or decrease the rate at which these antibodies are produced, be it because of T or B cell defects. Lack of insulin with resulting hyperglycaemia also decreases the ability of mast cells to degranulate and/or increases resistance of other cells to the action of mediators. Most importantly, however, insulin deficiency can abrogate an ongoing IgE response. This finding fits well with, and may be responsible for, the known clinical phenomenon that in asthmatic patients who develop diabetes the symptoms of allergy become less pronounced and occasionally vanish completely (Swern, 1932; Abrahamson, 1941; Szczeklik, Pieton & Sieradzki, 1980).

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REFERENCES

ABRAHAMSON, E.M. (1941) Asthma, diabetes mellitus and hyperinsulinism. *J. clin. Endocrinol.* **1,** 402.

ADAMKIEWICZ, V.W. (1963) Glycemia and immune responses. *Can. Med. Assoc. J.* **88,** 806.

ASHERSON, G.L. & PTAK, W. (1968) Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology*, **15,** 405.

ASKENASE, P.W., ROSENSTEIN, R.W. & PTAK, W. (1982) T cells produce an antigen binding factor with *in vivo* activity analogous to IgE antibody. *J. exp. Med.* (In press.)

BATES, G. & WEISS, C. (1941) Delayed development of antibody to anti-staphylococcus toxin in diabetic children. *Am. J. Dis. Child.* **62,** 346.

DOLKART, R.E., HALPERN, B. & PERLMAN, J. (1971) Comparison of antibody responses in normal and alloxan diabetic mice. *Diabetes*, **20,** 162.

FABRIS, N. & PIANTANELLI, N. (1977) Differential effect of pancreatectomy on humoral and cell-mediated immune responses. *Clin. exp. Immunol.* **28,** 315.

GANLEY, O.H. (1962) Studies on the prevention of sensitization by *Bordetella pertussis* in alloxan diabetic mice. *Can. J. Biochem.* **40,** 1179.

HELDERMAN, J.H. & REYNOLDS, T.C. (1978) The insulin receptor as a universal marker of activated lymphocytes. *Eur. J. Immunol.* **8,** 589.

HYVARINEN, A. & NIKKILA, B.A. (1962) Specific determination of blood glucose with *o*-toluidine. *Clin. Chim. Acta*, **7,** 140.

LIU, F.T., BOHN, J.W., FERRY, E.L., YAMAMOTO, H., MOLINARO, C.A., SHERMAN, L.A., KLINMAN, N.R. & KATZ, D.H. (1980) Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* **124,** 2728.

MALKIEL, S. & HARGIS, B.J. (1952) Anaphylactic shock in the pertussis-vaccinated mouse. *Allergy*, **23,** 352.

MICHAEL, J.G. & BERNSTEIN, I.L. (1973) Thymus dependence of reaginic antibody formation in mice. *J. Immunol.* **111,** 1600.

MOEN, J.K. & REIMAN, H.A. (1933) Immune reactions in diabetes. *Arch. Int. Med.* **51,** 1789.

OTTLECZ, A., KOLTAI, M., BLAZSO, G. & MINKER, E. (1978) Contributions to the regulatory role of insulin in inflammation and anaphylaxis. *Int. Arch. Allergy*, **56,** 284.

PEETERS, S.H. & CARTER, B.G. (1978) Regulation of the IgE antibody response in mice. I. Long term production of IgE anti-ovalbumin antibody in irradiated mice. *J. Immunol.* **121,** 1596.

PTAK, W., CZARNIK, Z. & HANCZAKOWSKA, M. (1975) Contact sensitivity in alloxan diabetic mice. *Clin. exp. Immunol.* **19,** 319.

PTAK, W., HANCZAKOWSKA, M. & ROZYCKA, D. (1977) Impaired antibody responses in alloxan diabetic mice. *Clin. exp. Immunol.* **29,** 140.

PTAK, W., REWICKA, M. & KOLLAT, M. (1980) Development of specific suppressor cells in hypoinsulinaemic mice. *Nature*, **283,** 199.

PTAK, W., REWICKA, M., KOLLAT, M. & WALICKI, W. (1981) Ease of induction of cells producing TNP-specific suppressor factor in hypoinsulinaemic mice. *Clin. Immunol. Immunopathol.* **19,** 447.

SANYAL, R.K., SPENCER, P.S.J. & WEST, G.B. (1959) Insulin and hypersensitivity. *Nature*, **184,** 2020.

SWERN, N. (19832) Incidence of diabetes mellitus in asthmatic patients. *J. Allergy*, **2,** 375.

SZCZEKLIK, A., PIETON, R. & SIERADZKI, J. (1980) Alteration in both insulin release and its hypoglycemic effects in atopic bronchial asthma. *J. Allergy*, **66,** 424.

THOMPSON, G.E. (1961) Alloxan and hypersensitivity. *Nature*, **190,** 822.