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Differential effect of pancreatectomy on humoral and cell-mediated immune responses

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SUMMARY

Cell-mediated immune reactions, such as allogenic skin-graft rejection and PHA or MLC responses, and antibody synthesis against different antigens (sheep erythrocytes, *Brucella* antigen, bovine serum albumin) have been evaluated in rats suffering from experimentally-induced diabetes and in age-matched sham-treated controls.

Cell-mediated immune reactions are strongly depressed in diabetic rats. The cellularity of the thymus and of thymus-dependent areas and the number of peripheral blood lymphocytes is significantly reduced in pancreatectomized rats. Moreover, the immunological recovery from heavy cortisonization is also greatly impaired. Daily treatment with insulin may prevent these immunological alterations.

By contrast, antibody responses in diabetic rats are not quantitatively altered in respect to either the number of antibody producing cells in the spleen or the circulating antibody titres.

The discrepancy between the abnormality of cell-mediated immune reactions in diabetic rats and their physiological capacity to synthetize antibodies suggests that the sensitivity to an insulin-deprived environment is present only in a definite, although yet undefined, subpopulation of lymphoid cells rather than in the whole lymphoid system.

INTRODUCTION

Recent investigations have pointed out that the efficiency of the immune system may depend on the endocrinological status of the animal (for review see: Fabris, 1976). Thus, it has been shown that developmental hormones, such as growth hormone, thyroxine and insulin, are needed for the ontogenetic maturation (Fabris, Pierpaoli & Sorkin, 1971a, b; Fabris, 1973a) and maintenance of the immune responsiveness throughout life (Fabris, 1973a; Duquensnoy, Mariani & Good, 1969; Pierpaoli, Fabris & Sorkin, 1970).

While the action of thyroxine seems to be exerted on both humoral and cell-mediated immune responses (Fabris, 1973a), the effect of growth hormone is preferentially directed on the T-dependent system and, particularly, on the proliferation rate of thymocytes within the thymus itself (Fabris *et al.*, 1971a, b, 1972; Pierpaoli *et al.*, 1970; Pierpaoli & Sorkin, 1972).

The relevance of insulin for the efficiency of the lymphoid system has been already suggested by some authors (Thompson, 1967; Pierpaoli *et al.*, 1971; Lundin & Angervall, 1970). It has been also shown that insulin stimulates ATPase activity and glucose uptake in human lymphocytes (Hadden *et al.*, 1972), and insulin receptors have been found on their membranes (Krug, Krug & Cuatrecasas, 1972; Archer, Gorder & Gavin, 1973).

With respect to immunological mechanisms, it has been reported that in diabetes mellitus humoral immune responses are normal (Dolkart, Halpern & Perlman, 1971), while PHA resposes are diminished (MacCuish Urbaniak & Campbell, 1974). In mice, alloxan induced diabetes causes impairment of

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contact sensitivity (Ptak, Gzarnik & Hanczakowska, 1975) and depressed transplantation immunity (Pierpaoli *et al.*, 1971).

The present paper, deals with findings on both humoral and cell-mediated immune responses evaluated in rats deprived either of the pancreas by means of surgical removal of the gland or only of insulin-producing cells by alloxanization (Riddick, Reisler & Kipnis, 1962).

Cell-mediated immunity has been tested by allogeneic skin graft survival, by unidirectional mixed leucocyte culture and by phytohaemagglutinin response. Humoral immune responses were evaluated by measuring the number of plaque-forming-cells against sheep erythrocytes (SRBC) and the serum antibody titres against bovine serum albumin (BSA) and *Brucella* antigen. In order to prove the insulin dependency of the immunological defects observed in pancreatectomized or alloxanized animals, immune responses have also been measured in diabetic rats daily treated with insulin.

MATERIALS AND METHODS

Animals. Inbred Sprague–Dawley (Charles River, Italy) male rats were used for the induction of experimental diabetes. Long–Evans rats, used as skin donors, and Brown Norway rats were kindly supplied by Roche (Basel).

Operative procedures. Pancreatectomy was performed under Thiogenal anestesia according to the method of Ingle & Griffith (1962). Sham-operated animals were used as controls. Operations were always on 30-day-old animals. Alloxanized animals were prepared by injecting i.p. 200 mg/kg body weight of Alloxan (Fluka) dissolved in saline. Such a drug did not seem to be toxic for lymphoid cells, since *in vitro* concentrations of alloxan ten times higher than those reached in the animal did not increase the number of dead thymocytes during 2-hr incubation period. The efficiency of the operation or of alloxan treatment was assessed by the appearence of glycosuria. Animals which did not become diabetic within 60 days after treatment were less than 10% and were discarded.

Ear skin from Long–Evans rats was transplanted onto the backs of pancreatectomized or sham-operated Charles River rats. Plastic corsets were removed 8 days after transplantation.

Hormonal preparation. Insulin (De Novo-Insulin, Novo-Industry, Copenhagen, Denmark) was diluted with physiological saline and injected subcutaneously in the amount of 0·1 iu/100 g body weight.

Hydrccortisone acetate (Fluka), used as an immunosuppressive drug, was dissolved in absolute alcohol and diluted with physiological saline in order to reach a solution containing 5% alcohol. Hydrocortison was injected s.c. in the amount of 40 mg/100 g body weight, 2 days before skin grafting.

Determination of spleen and thymus weights. Body weight was determined weekly and before excision of organs. The thymuses and spleens of rats were dissected free of fibrous connective tissue and weighed on a sensitive Mettler balance. The weights were recorded to nearest milligram.

Histological and haematological tests. Peripheral white blood cells (PWBC) were counted by bleeding the animals from the tail; differential counts were performed on smears stained with May-Grünwald-Giemsa. Lymphoid organs were fixed in Carnoy's fluid and stained with Haematoxylin and Eosine.

Humoral immune responses. Primary immune responses against sheep red blood cells (SRBC) were measured after one i.p. injection with 0·1 ml/100 g body weight of 20% erythrocyte suspension in saline. Haemagglutinins were measured on Takatsy plates. Plaque-forming-cells (PFC) were evaluated by plating $0{\cdot}5 \times 10^6$ spleen cells with 0·1 ml of SRBC suspension in agar Petri dishes, and adding, after incubation, lyophilized guinea-pig serum diluted 1:20.

Serum agglutinin titres against *Brucella* antigens were measured after one i.p. injection with 0·2 mg/100 g body weight of *Brucella* antigens (*Brucella* Trockenantigen–Max Pettenkofer Institut, Germany) suspended in saline. Agglutinins were measured on Takatsy plates by using a dyed preparation of the antigen.

PHA response. Spleen cells were obtained by teasing the spleen with fine forceps in cold Eagle's minimum essential medium (MEM). Further disruption was achieved by gentle aspiration with a Pasteur pipette. After sedimentation for 10 min the supernantant was centrifuged and the cells washed with Eagle's solution. Cells were finally resuspended and the number of viable ones determined by Trypan blue exclusion test. The cell suspension was diluted to 1×10^6 viable cells/ml with Eagle's minimum essential medium supplemented with vitamins (Gibco, Grand Island, New York), essential and non-essential amino-acids (Gibco), glutamine (Gibco) and 10% foetal calf serum (Wellcome batch no. 4251).

The cultures were incubated in 12×50-mm glass tubes at 2 ml/tube. Unless otherwise stated, 50 μl of PHA (Difco) was finally added. ^{3}H-labelled thymidine ([^{3}H]Td) (Amersham, sp. act. 5000 mCi/mM) was added in the amount of 0·5 μCi/tube at 48 hr of culture. Cells were harvested at 72 h of culture and the amount of radioactivity present in the trichloracetic acid precipitable material was measured in a Tri-Card (Packard) liquid scintillation counter.

Unidirectional mixed leucocyte reaction. 1×10^6 thymus or spleen cells, obtained as reported above, were distributed in 12×50 glass tubes for mixed cultures, a double amount being used for control cultures. Spleen cells from (Charles River $\times$ Brown Norway) F_1 hybrid rats were used as stimulator cells. They were mixed with parental cells in the amount of 2×10^6 cells contained in 1 ml of culture medium. ^{3}H-labelled thymidine was added to mixed and control cultures 22 hr before the rats were killed. Cells were harvested the 5th day and [^{3}H]Td uptake was evaluated as reported above.

RESULTS

Body growth is significantly impaired in either pancreatectomized or alloxanized rats. The maximal body weight reached by diabetic rats ranges between 150–200 g while sham-operated or untreated controls weighed from 300 to 350 g. Life-span is shortened: 6–10 months in diabetic rats, 20–28 months in sham-operated controls. Diabetic rats treated daily with insulin at a dosage which prevents glycosuria grew normally and their life-span was prolonged; the extent, however, depended on the length of hormonal therapy.

Since the immunological disturbances observed in alloxanized rats did not differ from those present in pancreatectomized animals, the data reported below are based primarily on pancreatectomized rats. When data on alloxanized animals are presented, they will be specified.

(a) *Progressive decrease of circulating lymphocytes*

The number of peripheral white blood cells (PWBC) was evaluated in groups of ten pancreatectomized

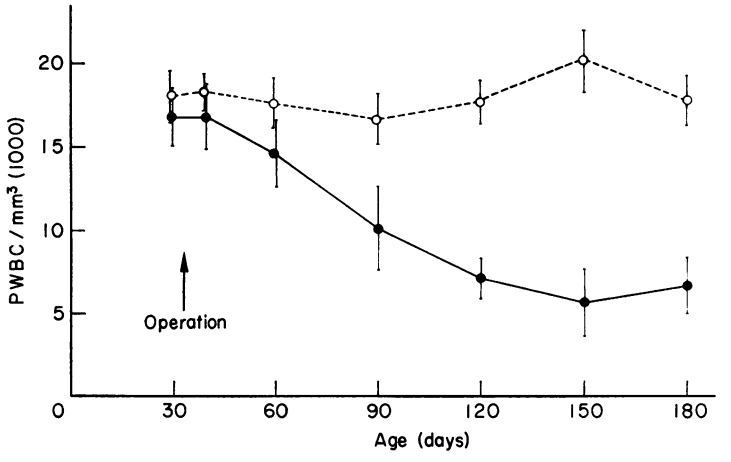

FIG. 1. Progressive reduction of PWBC number in pancreatectomized rats (●——●) as compared with sham-operated controls (○- -○) at different time after operation. Each point represents the mean and s.e. of 8–10 rats per group.

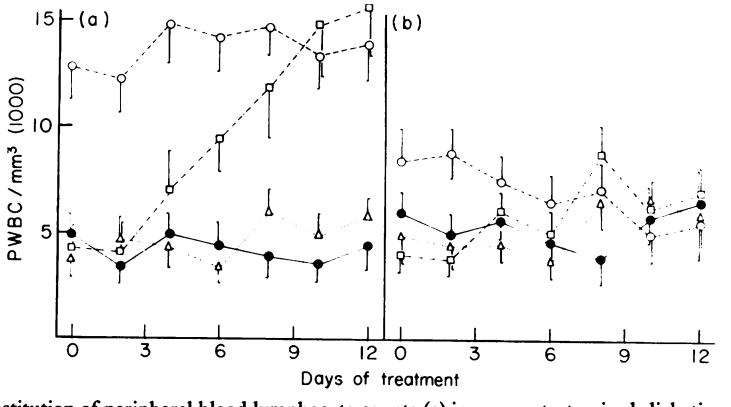

FIG. 2. Reconstitution of peripheral blood lymphocyte counts (a) in pancreatectomized-diabetic rats by injections with insulin at the daily dose of 0·1 iu/100 g body weight (□–·–·–□). Saline treated diabetic rats (●——●); diabetic rats treated with growth hormone (GH) at the daily dose of 100 μg/100 g b.wt (△·····△); saline-treated sham-operated rats (○ – – –○). To note that the number of polymorphonucleated cells (b) are not modified by pancreatectomy or hormonal treatments. Each point represents the mean and s.e. of 8–10 rats per group.

and ten sham-operated animals, the blood being withdrawn from the tail at 10.00 a.m. without previous fasting. Fig. 1 shows the progressive reduction of the number of PWBC observed in pancreatectomized rats when compared with sham-operated animals. The reduction starts to be statistically significant 60 days after the operation, when pancreatectomized rats show an overt diabetic condition as revealed by glycosuria.

The maximal reduction of the PWBC number is reached 90 days after the operation, in the following periods no further reduction being observable. Differential counts performed on smears of peripheral blood withdrawn the 90th day after the operation reveal that the reduction of the PWBC number is mainly due to a decreased lymphocyte population rather than to polymorphonucleated cells, which do not appear to be greatly modified by pancreatectomy (Fig. 2).

The percentage of peripheral lymphocytes in respect to the total number of PWBC also remains low but constant in pancreatectomized rats in the following period of life. Even diabetic rats surviving up to 250 days show a persistent lymphocyte population constituting the 40–50% of total PWBC counts.

Moreover one injection of hydrocortisone acetate at dosage of 40 mg/100 g body weight does not further reduce the number of peripheral lymphocytes in pancreatectomized rats, whereas a profound decrease is observed on sham-operated rats (Table 1), suggesting that the majority of lymphocytes present in diabetic rats are relatively insensitive either to steroids or to the pancreatectomy-conditioned internal environment.

TABLE 1. Number of peripheral lymphocytes in pancreatectomized or sham-operated rats receiving one injection with hydrocortisone (HC)

Days after HC*	No. of peripheral lymphocytes/mm^3	
	Pancreatectomized	Sham-operated
0	5·750± 790	15·560± 1·020
1	3·370± 375	5·900± 910
3	3·740± 840	6·380± 560
5	4·120± 755	7·930± 1·050
7	3·910± 335	9·210± 960

* Hydrocortisone acetate was injected once at the dose of 40 mg/100 g body weight in animals operated 60 days before. Each number represents the mean and s.e. of six rats, each independent value being the mean of the PWBC counts recorded in the same animal in two consecutive blood withdrawal (30-min interval).

Daily treatment of diabetic rats (beginning at 90 days after the operation) with insulin induces a striking increase in the number of circulating lymphocytes, so that normal values are reached in about 9 days of treatment (Fig. 2). Treatment with saline or with growth hormone, which has the capacity to increase peripheral blood lymphocytes in hypopituitary dwarf mice (Fabris *et al.*, 1971a and b), does not have any effect (Fig. 2).

(b) *Early thymus involution in pancreatectomized rats*

Both the absolute and the relative weights of the thymus are significantly reduced in pancreatectomized rats when compared to the values observed in age-matched sham-operated controls (Fig. 3). Such a reduction is observable in all experimental groups sacrificed at more than 30 days after the operation, whereas at 15 days after the operation there are no differences in thymus weights between pancreatectomized and sham-operated rats. By contrast, the spleen of pancreatectomized rats shows normal weights according to the age of animal at the time of killing (Fig. 3).

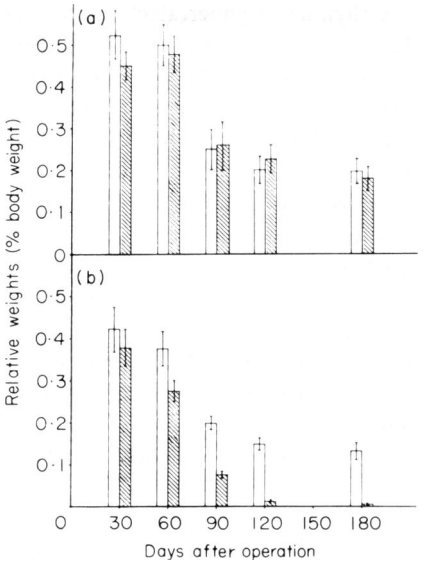

FIG. 3. Progressive reduction of relative weights of the thymus in pancreatectomized rats (▨) when compared to age-matched sham-operated controls (□), at different time after the operation. Spleen weights (a) are not modified by pancreatectomy. Each point represents the mean and s.e. of 4–6 rats per group.

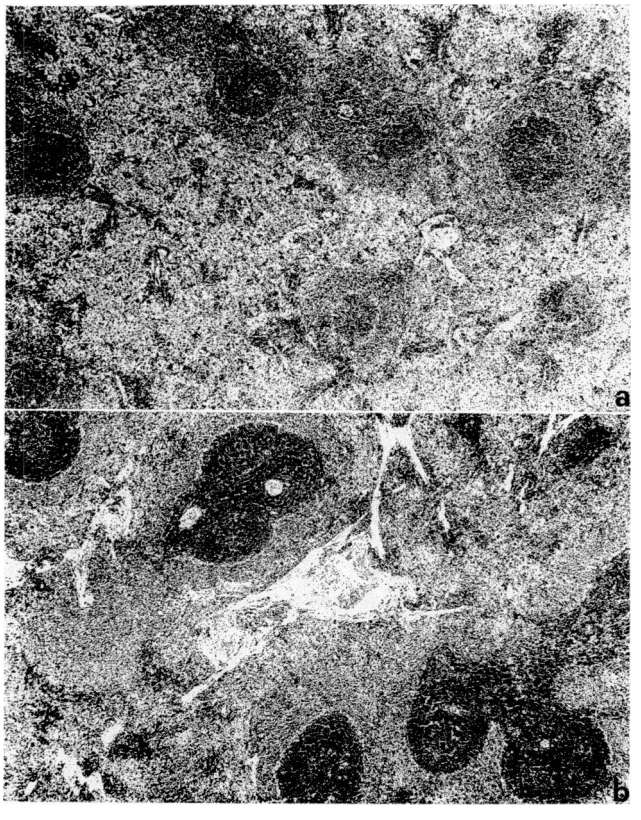

FIG. 4. Pronounced depletion of the follicular mantel of small lymphocytes in the spleen of pancreatectomized-diabetic rats (a) when compared with sham-operated controls (b), at 90 days after intervention. (H & E × 38.)

The histological structure of the thymus in pancreatectomized rats appears normal, although the cellularity of the organ is generally reduced. No signs of cell death have been observed.

On the other hand the structure of the spleen of pancreatectomized rats is radically altered: the lymphoid follicles are characterized by a pronounced depletion of the perifollicular mantel of small lymphocytes (Fig. 4a and b). Also the periarteriolar sheets show a reduced cellularity. Germinal centers, on the contrary, are well developed.

Preliminary experiments on lymphoid cell turnover by evaluating ^{3}H-labelled thymidine labelled cells in squash preparations of thymus and spleen from animals injected with [^{3}H]Td 24 hr before killing, support the findings reported above.

The thymus of pancreatectomized rats show a 75% reduction in labelled cells when compared to the thymus of age-matched sham-operated controls (56 labelled nuclei ‰ cells in pancreatectomized rats; 243‰ in sham-operated controls). In contrast the percent of labelled cells, in spleen squashes from pancreatectomized rats is similar to that observed in sham-operated controls (103‰ in pancreatectomized rats *vs* 88‰ in sham-operated animals).

These observations would indicate that the cell turnover in the thymus or in the spleen of pancreatectomized animals is different.

(c) *Humoral immune responses in pancreatectomized rats*

Pancreatectomized rats challenged with sheep erythrocytes, i.p. injected in the amount of 0·1 ml/100 g body weight of a 20% SRBC suspension in saline, form as many PFC as sham-operated controls.

The kinetic of the direct primary PFC response reveals, in fact, no substantial differences between the two experimental groups, either in the number of PFC per million spleen cells, or in the time needed to reach the maximal peak (Fig. 5).

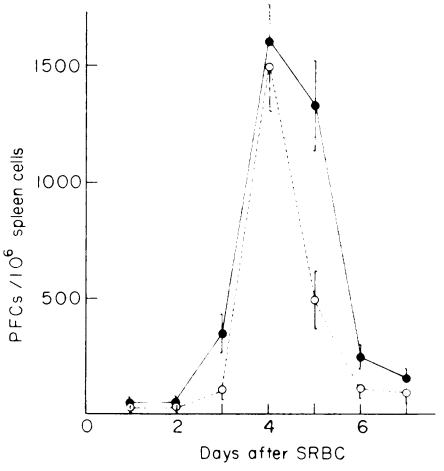

FIG. 5. Primary plaque-forming-cell response in pancreatectetomized (●——●) and in sham-operated (○- - - - -○) rats, after challenge with 0·1 ml/100 g b.wt. of a 20% SRBC suspension in saline. Each point represents the mean and s.e. of four to six rats per group.

The 4th day peak of PFC response is normal in pancreatectomized rats in all, but one, age-groups tested, this in spite of the presence of hyperglycaemia revealed by glycosuria (Fig. 6).

In the oldest group of pancreatectomized rats challenged with SRBC, i.e. at 150 days after the operation, the 4th day peak is reduced. Such a reduction is probably due to the general cachexia, which frequently affects diabetic rats in the last stages of their life, although diabetic rats of the same age and in similar condition respond quite well to booster injections with other antigens, such as BSA (see below). The haemagglutinin titres evaluated in animals pancreatectomized 60 days before immunization, do not show

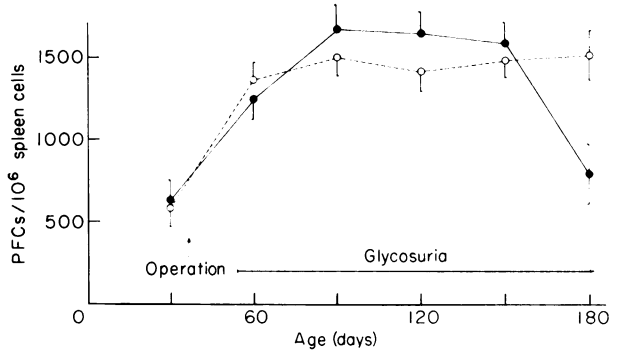

FIG. 6. Plaque-forming cell capacity, as measured by the 4th day peak response, in pancreatectomized (●——●) and in sham-operated (○- - - -○) rats, challenged with 0·1 ml/100 g b.wt. of a 20% SRBC suspension in saline at different time after operation. Each point represents the mean and s.e. of four to six rats per group.

TABLE 2. Agglutinin responses to SRBC or *Brucella* antigen in sham-operated and in pancreatectomized or alloxanized young adult rats

Days after immunization	Agglutinin titres*				
	Anti-SRBC†		Anti-*Brucella*†		
	Pancreatectomized	Sham-operated	Pancreatectomized	Alloxanized‡	Sham-operated
2	1:10	1:10	—	—	—
3	1:40	1:20	Negative	1:10	1:10
4	1:640	1:320	—	—	—
5	1:5120	1:640	1:320	1:320	1:160
6	1:2560	1:640	—	—	—
7	1:5120	1:640	1:640	1:1280	1:640

* Each value represents the titre of pooled sera from five animals treated with identical experimental schedule.

† Immunization against SRBC was achieved by injecting 0·1 ml/100 g body weight of a 20% SRBC suspension in saline. Immunization against *Brucella* antigen was achieved by injecting 0·2 mg/100 g body weight of *Brucella* antigen suspended in saline.

‡ Alloxan-diabetes was induced by an i.p. injection with 200 mg/kg b.wt. of Alloxan dissolved in saline.

significant differences when compared to those observed in sham-operated litter mates, although somewhat higher values have been found in pancreatectomized than in sham-operated animals (Table 2).

Similar findings have been obtained with other antigenic challenges. Thus agglutinin response against Brucella antigens is normal in pancreatectomized as well as in alloxanized rats (Table 2).

Immunization with BSA in Freund's complete adjuvant into the footpads induced similar antibody responses in alloxanized or untreated rats (Table 3). Moreover secondary or tertiary antibody responses, elicited by booster injections with BSA in saline at 30 or 90 days after primary challenge, are quantitatively similar in both experimental groups (Table 3).

(d) *Delayed skin-graft rejection in pancreatectomized rats*

The capacity to reject allogeneic skin-grafts is significantly impaired in rats pancreatectomized 60 days before grafting (Fig. 7). Allogeneic skin-grafts survive 18–20 days (mean rejection time = 19·0 days) in pancreatectomized animals, while in sham-operated littermates they survive 9–12 days (mean rejection time = 10·7 days). Such a delay is even more pronounced when animals are treated, 2 days before grafting, with an immunosuppressive drug such as hydrocortisone acetate at the dose of 40 mg/100 g body weight.

TABLE 3. Humoral immune responses against bovine serum albumin (BSA) in alloxanized-diabetic or untreated rats

Immune response	Days after first challenge†	Days after booster challenge†	Agglutinin titres* Allox.	Agglutinin titres* Untreated
	7	—	Negative	Negative
Primary	13	—	1:64	1:32
	19	—	1:256	1:256
Secondary	33	2	1:1280	1:2560
Tertiary	90	2	1:2560	1:2560

* Each value represents the titre of pooled sera from five animals treated with identical experimental schedule.

† First antigenic challenge was achieved by injecting into footpads 0·5 mg of BSA in Freund's complete adjuvant. For booster challenges a s.c. injection with 0·5 mg BSA dissolved in saline was used.

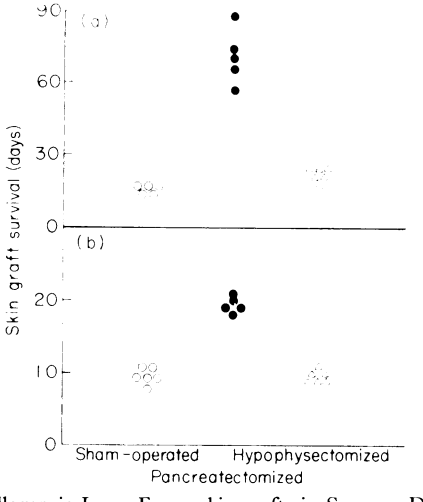

FIG. 7. Delayed rejection of allogeneic Long–Evans skin grafts in Spraque–Dawley pancreatectomized rats (●) when compared with sham-operated controls (○) or hypophysectomized animals (△). One s.c. injection with hydrocortisone at the dose of 40 mg/100 g body weight 2 days before skin grafting (upper part of the figure) shows that immunological recovery is strongly impaired in pancreatectomized rats. Skin grafts were always performed 60 days after the operation. Each point represents one animal. (a) with cortisone; (b) without cortisone.

In these experimental conditions, heavily cortisonized sham-operated rats do not show significant prolongation of skin-graft survival (mean rejection time = 11·2 days) whereas similarly treated pancreatectomized rats keep the graft for 54–91 days (mean rejection time = 70·2 days). Thus, not only the actual capacity to mount the reaction against allogeneic skin-grafts is impaired in pancreatectomized rats, but also the immunological recovery from heavy cortisonization.

The specific dependence of such an immunological impairment from the pancreas is demonstrated by the fact that the removal of other endocrine glands, such as the hypophysis (Fig. 7) or the thyroid which are as stressfull as pancreatectomy, do not impair the skin-graft rejection capacity, at least to the extent observed in pancreatectomized animals.

(e) *Reduced in vitro blastic transformation of lymphocytes from pancreatectomized rats*

Spleen or thymus lymphocytes obtained from diabetic animals (60 days after the operation) have

TABLE 4. Reactivity of spleen and thymus cells from pancreatectomized or sham-operated Charles River rats to PHA or to (Charles River × Brown Norway) F_1 hybrid spleen cells

Responder cells*	Stimulator cells*	Ct/min/culture†
Pancreatectomized spleen	F_1 spleen	2·780 ± 236
Sham-operated spleen	F_1 spleen	4·916 ± 380
Pancreatectomized thymus	F_1 spleen	1·978 ± 260
Sham-operated thymus	F_1 spleen	3·668 ± 312
Pancreatectomized spleen	PHA	20·317 ± 2·650
Sham-operated spleen	PHA	38·380 ± 3·155
Pancreatectomized thymus	PHA	1·652 ± 365
Sham-operated thymus	PHA	359 ± 160

Animals at 60 days after the operation were used as donors of responder cells.

* Radioactivity in unstimulated culture was below 450 ct/min/culture and it has been subtracted.

† Each value represents mean ± s.e. of five animals.

been tested *in vitro* against allogeneic F_1 hybrid spleen cells or PHA and the results compared with those obtained in sham-operated controls.

The reaction of both spleen and thymus lymphocytes from pancreatectomized rats against allogeneic F_1 hybrid spleen cells, evaluated on the 5th day of culture, is lower than that mounted by lymphocytes from sham-operated littermates, the reduction ranging between 30–50% of normal value (Table 4). The PHA response of spleen lymphocytes from pancreatectomized rats, evaluated on the 3rd day of culture, is reduced, when compared to normal values (Table 4), whereas the PHA response of thymocytes from pancreatectomized rats is higher than that of thymocytes from sham-operated rats.

DISCUSSION

The most relevant finding of our work is that insulin deficiency, while irrelevant for antibody synthesis, and this in spite of the known effect of insulin on the energy metabolism and protein synthesis, strongly impairs the proliferation and, may be, even the differentiation of lymphocytes involved in cell-mediated immunity. This assumption is supported by the observation that the majority of the morphological and functional aspects of the thymus dependent compartment such as the absolute and relative weight of the thymus, the cellularity of thymus-dependent areas, the allogeneic skin-rejection capacity, the PHA response and the reactivity in the unidirectional leucocyte culture are significantly depressed in pancreatectomized animals when compared to control littermates.

Such an immunological deficiency seems to stem from an inadequate supply of reactive lymphocytes by the thymus rather than from the actual effect of the internal environment.

This interpretation is supported by the existence of peripheral lymphopenia, of hypoplasia of thymus-dependent areas, and of decreased PHA and MLC reactions in pancreatectomized rats, although these latter findings are partially limited by the fact that a single *in vitro* incubation time and a single concentration of allogeneic cells or PHA have been used.

The higher PHA reactivity of thymocytes from pancreatectomized rats as compared to that of thymocytes from sham-operated controls, may further support the diminished production of mature T-cells by the thymus of pancreatectomized rats since it indicates a contraction of the PHA-insensitive cell population, similarly to what happens, for instance, after steroid treatment (Cohen & Mosier, 1974).

With regard to transplantation immunity it is to be noted however, that the delayed rejection of

allogeneic skin graft in pancreatectomized rats may also be due to an interference by cells with suppressor activity (Katz *et al.*, 1974), or other mechanisms of immunological rejection (anti-graft antibodies), whose function according to our results, should not be altered by pancreatectomy.

It is quite surprising, on the other hand, that antibody responses to antigens, which are known to be thymus-dependent, such as SRBC (Miller & Mitchell, 1969), or BSA (Taylor, 1969), are normal in pancreatectomized rats. We do not have a clear explanation accounting for these findings, although it is not unlikely that subpopulations of thymus-derived cells may either take a different length of time to become exhausted in an insulin-deprived environment or be totally resistant because of lack of hormone receptors. While the absence of insulin receptors on the membrane of a still undefined population of peripheral lymphocytes has been documented (Olefski & Reaven, 1974), no data are available on subpopulations of thymocytes.

The assumption that insulin deficiency is the primary cause of the observed effects in pancreatectomized or alloxanized animals, stems from the experimental possibility, on one hand, to reproduce the immuno-deficiency induced by pancreatectomy by treating rats with alloxan, a drug which destroys only insulin-producing cells while leaving unaffected the synthesis of other pancreatic humoral factors; and on the other hand, to restore completely the immunological capacity of both pancreatectomized or alloxanized animals by daily injections with insulin.

With regard to the mode of action of insulin on the lymphoid system, the present data do not answer the question whether the effect of insulin is direct or mediated through the known insulin-dependent metabolic alterations. It has been suggested that hyperglicaemia '*per se*' may reduce PHA responses in human lymphocytes (MacCuish *et al.*, 1974). The observation, however, that exogenous administration of insulin to otherwise untreated rats increases the magnitude of tuberculin reaction (Thompson, 1967) may, at least, indicate a close physiological relationship between glucose levels and cell-mediated immunity. On the other hand the demonstration of insulin receptors on the membrane of peripheral lymphocytes (Krug *et al.*, 1972; Olefski & Reaven 1974) favours the hypothesis of the direct effect.

Notwithstanding the oversimplification of our experimental model it seems justified, however, to deduce that insulin, directly or indirectly, may be considered an expanding factor for T lymphocytes involved in cell-mediated immunity. Such a deduction introduces the concept that hormones, instead of being aspecific factors merely acting in order to enhance or depress the immunological reactivity, may selectively interfere with the proliferation rate of definite populations of lymphoid cells.

This assumption is supported by observations other than those regarding insulin (Fabris, 1976).

Growth hormone, for instance, seems to be more relevant to the ontogenetic development and for the maintenance of the thymus-dependent reactivity rather then to the efficiency of the B-dependent system (Fabris *et al.*, 1971; Pierpaoli *et al.*, 1970; Arrembrecht & Sorkin, 1973). The demonstration of specific membrane receptors for growth hormone on thymocytes but not on peripheral lymphocytes supports this view (Arrembrecht, 1974).

On the other hand some still undefined pregnancy hormones may be considered as expanding factors for cells involved in antibody synthesis as demonstrated by the abnormally increased PFC capacity recorded in pregnant normal mice (Fabris, 1973b) and in pregnant nude mice (Rowley, personal comunication), as well as in female nude mice treated with human chorionic gonadotrophin.

These data, taken together, suggest that subpopulations of lymphoid cells may require different hormonal balances in order to fully express their potential, or, adversely, that the physiological endocrine balance (or the metabolic conditions deriving from it) may contribute to the homeostatic control among various immunological functions. Such an idea agrees with the hormone sensitivity of lymphoid tumours.

Thus hypophysectomy prevents the emergence of Gross virus-induced leukaemia in rats (Bentley, Hughes & Peterson, 1974), treatment with anti-pituitary antiserum decreases the incidence of X-ray or DMBA induced lymphosarcomas in mice (Pierpaoli & Haran-Ghera, 1975), while pituitary gonadotropines and testosterone enhance the incidence and the growth rate of mineral-oil induced myeloma in mice (Hollander *et al.*, 1968).

On the other hand the concentration of insulin receptors seems to be higher in lymphoblastoid cells than in peripheral lymphocytes (Krug *et al.*, 1972).

If so, it is not inconceivable, although speculative at present, that during the life of an organism, hormonal conditions which favour abnormal cloning of lymphoid cells, may arise only in some periods of life.

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