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Pancreatic lesions induced in rabbits and guinea-pigs with pancreatic antigens

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SUMMARY

Rabbits and guinea-pigs were immunized with various pancreatic antigens in Freund's adjuvant. Rabbits received unfractionated bovine insulin and the 'A' component and 'single peak' insulin separated from it by gel-filtration. All produced antibodies capable of reacting with porcine insulin but none were found to have pancreatic lesions when killed up to 6 weeks after initial injection. Guinea-pigs immunized with bovine 'A' component developed pancreatic peri-ductulitis which appeared most frequently (10/20) in animals killed 30 days after a single injection and less frequently in animals killed after 60 (4/10) and 90 (1/10) days. Similar lesions were found in only a small proportion of control animals (2/23) or of guinea-pigs immunized with single peak bovine insulin (3/22). Guinea-pigs immunized with homogenates of homologous and heterologous islets of Langerhans developed signs of peri-ductulitis in a high proportion of animals killed up to about 60 days after first injection (18/26). None of these animals exhibited clearly defined signs of diabetes mellitus and the incidence of induced lesions could not be correlated with levels of circulating insulin-binding antibodies.

INTRODUCTION

When the present investigations were first contemplated, two reported phenomena seemed of particular interest. First, Renold *et al.* (1966) had observed lymphocytic infiltration of the islets of Langerhans in cattle which had been repeatedly injected with bovine or porcine insulin in Freund's adjuvant. Similar lesions have since been reported in rabbits (Kloppel, Altenahr & Freytag, 1972; Kloppel *et al.*, 1974; Lee *et al.*, 1969; Toreson, Lee & Grodsky, 1968), mice (Freytag, Jansen & Kloppel, 1973; Jansen *et al.*, 1974) and sheep (Federlin, 1971) immunized with various insulin preparations. These experimentally induced lesions appeared to be very similar to those sometimes seen in the early stages of juvenile diabetes by Gepts (1965), LeCompte (1958), Maclean & Ogilvie (1959), Warren, LeCompte & Legg (1966) and others. Secondly, and following an initial observation by Mirsky & Kawamura (1966), it is now well known that crystalline preparations of insulin contain not only the hormone itself but also proinsulin, derivatives of proinsulin and insulin, and a constituent of high molecular weight which is distinctly heterogeneous (Steiner & Oyer, 1967). This latter constituent, which will hereafter be termed 'A' component, has been shown to be highly immunogenic in rabbits and is currently thought by some to be the immunogenic constituent of many commercial preparations of insulin (Schlichtkrull *et al.*, 1972). The present studies were carried out to determine whether cellular changes could be induced in the pancreatic tissues of rabbits or guinea-pigs with insulin itself, contaminating 'A' component or with homogenized preparations of islet tissue. Attention was concentrated on the early immune responses of these animals because others had found such experimental lesions within 2 or 3 weeks.

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MATERIALS AND METHODS

Antigens. Derivatives of bovine insulin consisted of the unfractionated crystalline hormone (Lot No. T-2842) and of the 'single peak' (lot nos 615-1070B-210-4 and 615-1112B-160) insulin and 'A' component (lot no. 615-1082B-194-A-1) derived from it by gel-filtration on G-50 Sephadex columns (Steiner & Oyer, 1967). Unlabelled (lot no. PJ-5682) and ^{125}I-labelled (*c* 100 μCi/μg; New England Nuclear, Boston, Massachusetts) porcine insulins were used for the assay of insulin binding antibodies in sera (Makulu & Wright, 1971).

Isolated islets of Langerhans were obtained in quantity from male albino rats (200–300 g) and from male albino guinea-pigs (500-800 g) by modifications of the methods described by Lacy & Kostianovsky (1967) and by Lindall, Steffes & Sorenson (1969). After inflation with injected buffered Hanks's solution, the pancreas was cut into small pieces and incubated at 36°C for 5–12 min with collagenase (Type IV; Worthington Biochemical Corporation, Freehold, New Jersey). The resultant digest, after being washed twice with buffer to remove excess collagenase, was suspended in a concentrated solution of Ficoll (30%, w/v; (Pharmacia Fine Chemicals, Uppsala, Sweden). Above this concentrated suspension (*ca* 30 ml) in a large centrifuge tube (27 × 115 mm), solutions of Ficoll were layered in decreasing concentrations (5–10 ml; 27, 25, 23, 21, 19 and 17%, w/v). Islets and acinar tissue elements separated during centrifugation (10 min; 800 *g*) as illustrated in Fig. 1. Pooled pancreatic tissue from four rats yielded varying amounts of islet tissue (78–266 μg islet protein/rat), the islets separating almost free of acinar tissue elements below the interfaces separating the 17, 19 and 21% solutions of Ficoll. One or two medium-sized guinea-pigs yielded more but still very variable quantities of islet tissue (203–5440 μg islet protein/guinea-pig), the islets separating in increasing sizes below the interfaces separating (respectively) the 17, 19, 21 and 23% solutions of Ficoll. No satisfactory separation of rabbit islets could be achieved by this method because acinar tissue elements contaminated all layers in which islets could be seen. Islets were collected in siliconized Pasteur pipettes from the layers of Ficoll in which they had separated free of obvious acinar tissue elements, were washed and were then finally suspended in buffered Hanks's solution.

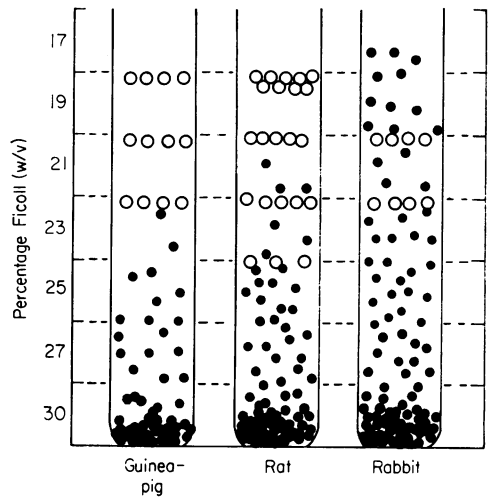

FIG. 1. Separation of islets of Langerhans (○) from acinar elements (●) of pancreatic tissue from guinea-pigs, rats and rabbits. Collagenase digests of pancreatic tissue were suspended in a concentrated solution (30%, w/v) of Ficoll and then covered with layers of Ficoll solutions in decreasing concentrations (27–17%, w/v). The figure shows the separations achieved after subsequent centrifugation for 10 min at 800 *g*.

Immunization procedures. In a total of four experiments, rabbits and guinea-pigs were immunized with bovine insulin preparations and/or homogenates of islets in water-in-oil emulsions. All antigens were dissolved or suspended in an aqueous phase (10 volumes) which was then emulsified with an equal volume of an oily phase composed of heavy mineral oil (7 volumes, U.S.P.) and lanolin (3 volumes; Adeps lanae, anhydrous, U.S.P.). Included in the first but omitted from all subsequent injections given to any individual animal were either Pertussis vaccine (3 volumes, fluid, U.S.P., Eli Lilly Company, Indianapolis, Indiana) added to the aqueous phase (7 volumes) or killed dry *M. butyricum* (4 mg/inoculum; Difco Laboratories, Detroit, Michigan) added to the oily phase before emulsification. Injections were given into the foot pads on the hind legs of rabbits for the first inoculum. All subsequent injections into rabbits and all injections given to guinea-pigs were given subcutaneously into the lower abdominal wall.

Experiment 1. Male albino rabbits (mean body weight, 2012 ± 28 g) were divided into groups of five (control) or fifteen (immunized) for immunization with bovine 'A' component or with 'single peak' and unfractionated bovine insulins. The inocula given to each animal (0·2 or 0·4 ml emulsion/inoculum) contained the antigens (1·0 mg) dissolved in dilute hydro-

chloric (0·01 N) or acetic (0·6% glacial, v/v) acid and, for the first injection only, *Pertussis* vaccine. Of the fifteen animals in each group of immunized rabbits, ten received only one injection of antigen on the first day (day 0) and were killed 20 days later, and five were injected twice with antigen on days 0 and 21 and then killed on day 40. The control animals were injected on days 0 and 21 with antigen-free emulsions and killed on day 40.

Experiment 2. A small group of ten rabbits (mean body weight, 2106± 138 g) was injected at weekly intervals with emulsions (2·0 ml/inoculum) containing unfractionated bovine insulin (1·0 mg) dissolved in dilute acetic acid and, for the first injection only, killed dry *M. butyricum*. Following initial injection (day 0), five rabbits were killed on day 22 after receiving a total of three inocula and the remainder were killed on day 35 after receiving a total of five inocula.

Experiment 3. Groups of eight to twenty male albino guinea-pigs weighing between 300 and 450 g were injected with emulsions (2·0 ml/inoculum) containing no antigen, bovine 'single peak' insulin or bovine 'A' component. The antigens (1·0 mg/inoculum) were dissolved in dilute (0·01 N) hydrochloric acid and the first inoculum contained *Pertussis* vaccine. Following the first injection (day 0), groups of guinea-pigs were killed on day 30 without additional injections, on day 60 after one additional injection of antigen on day 27, or on day 90 after two additional injections of antigen on days 27 and 60.

Experiment 4. Groups of three to nine male albino guinea-pigs weighing between 300 and 450 g were injected with emulsions (1·0 ml/inoculum) containing homogenates of rat or guinea-pig islets in saline. The doses (mean± s.e.m., μg islet protein per inoculum) of rat (135± 10) and guinea-pig (781± 156) islet protein varied considerably, mainly because of improving yields obtained during successive isolations. Controls animals received no injections ($n = 6$), homogenates of rat acinar tissue recovered from the most concentrated solutions of Ficoll ($n = 3$) or emulsions containing no added antigens ($n = 5$). Pertussis vaccine was added to the initial inocula. Following this first injection on day 0, groups of animals were killed on days 20 or 21 after no additional injections, on days 38–41 after an additional injection on day 21, or on days 56–62 after two additional injections on days 21 and 42.

Rabbits were killed under anaesthesia (pentobarbital) by exsanguination. Guinea-pigs were either decapitated (experiment 3) or exsanguinated under anaesthesia (pentobarbital) when blood was drawn from the aorta or heart (experiment 4).

Experimental observations. Of the sixty rabbits used in the first experiments (Nos 1 and 2), three either died or had to be killed; one developed coccidiosis, one broke its leg during hypoglycaemic convulsions and the third was found dead about 18 hr after its second injection of insulin. Of 128 guinea-pigs used in the last two experiments (Nos 3 and 4), three were found dead in their cages, two being controls and the third having received injections of guinea-pig islets. All animals had free access to food and water at all times. The following specific investigations were carried out.

(a) *Blood and serum.* Blood obtained from rabbit's ears during life and from all animals at the time of killing was used to determine blood-sugar concentration (Reflectance Meter, Ames Company, Elkhart, Indiana). From blood collected at the time of killing, serum was separated and stored in the frozen state. Insulin-binding capacities of all such sera were measured by slight modifications of a method already described in detail (Makulu & Wright, 1971). Rabbit sera (50 μl) were incubated at 4°C for 2 days with a mixture of unlabelled (40 micro-units (μu)) and ^{125}I-labelled (*c* 10 μu) porcine insulins in a diluent buffer (0·3 ml). Sera from guinea-pigs (0·1 ml) were first diluted in buffer (4·9 ml), the diluted sera (50 μl) then being incubated for 18–24 hr at 4°C with mixtures of unlabelled (0·25–2·00 milli-units (mu)) and ^{125}I-labelled (*c* 10 μu) porcine insulins in buffer (0·3 ml). In each case, antibody-bound labelled insulin was precipitated with alcohol (1·4 ml ethanol, 95%, v/v), the precipitate washed with more dilute alcohol (2·0 ml, 76%, v/v), and its gamma-radioactive content determined in an automatic well-type scintillation counter (Packard Instruments Company, LaGrange, Illinois). The diluent buffer used in this assay consisted of a neutral phosphate buffer (0·05 M, Na_2HPO_4/KH_2PO_4; pH 7·0) containing added sodium chloride (0·4%, w/v) and normal guinea-pig serum (3%, v/v). Potencies after correction for appropriate blanks, are stated as percentages of total added immunoreactive labelled hormone bound by the volumes of sera indicated above.

(b) *Urine.* All urine specimens obtained from bladders at times of killing were tested for the presence of glucose and ketones (Keto-diastix, Ames Company, Elkhart, Indiana).

(c) *Tissues.* Portions of several tissues (liver, kidneys, spleen, pancreas, lymph nodes, etc.) were removed rapidly after and fixed in buffered neutral formalin and in Helly's Fluid (Formol Zenkers). Sections of these tissues were subsequently stained with haematoxylin and eosin (H & E) with Giemsa's stain (G) and, in the case of the pancreas only, with aldehyde–fuchsin (A–F).

RESULTS

Rabbits

With the exception of the three rabbits which died or had to be killed (see above), all animals remained healthy and gained weight. Signs of hypoglycaemia (flaccidity, convulsions, etc.) were noted in three animals about 2 hr after their first injection of insulin but all responded well to intraperitoneal injections of glucose. No animal showed clear evidence of persistent hyperglycaemia or other overt signs of diabetes mellitus during life. At times of killing no glucose was ever found in urine taken from the bladder.

At necropsy, small granulomatous lesions were found between the metatarsal bones of the hind feet of rabbits used in the first experiment (No. 1) but in the second group of animals (Expt 2) which received *M. butyricum* in the initial inoculum large and sometimes ulcerating lesions were nearly always

found at these injection sites. No other gross lesions could be found in any of the animals used in either experiment. In sections of pancreatic tissue, small collections of eosinophils were occasionally seen in the immediate vicinity of large pancreatic ducts and in interstitial spaces between acinar exocrine cells. Very occasional round cells were also seen. None of these cells were ever seen in or adjacent to islets of Langerhans which showed normal degrees of β-cell granulation.

As shown in Table 1, sera from rabbits immunized with unfractionated insulin or bovine 'A' component bound as much labelled insulin as those from animals immunized with 'single peak' bovine insulin.

TABLE 1. Labelled insulin binding by sera and pancreatic lesions of rabbits immunized with bovine insulin and bovine 'A' component

	Immunization programme*			Necropsy findings				
Expt No.	Antigen	Injections given	Killed	Animals (*n*)	Blood-sugar (mg/100 ml)	Labelled insulin binding by serum (%/50 μl)	Pancreatic lesions	
							Present	Absent
1	Nil	Days 0 and 20	Day 40	5	128±4	0	0	5
	Single peak insulin	Day 0	Day 20	9	155±19	45·4±6·5	0	9
		Days 0 and 20	Day 40	4	129±10	46·5±9·9	0	4
	Unfractionated insulin	Day 0	Day 20	9	116±9	43·5±4·0	0	9
		Days 0 and 20	Day 40	5	144±7	47·8±5·5	0	5
	'A' component	Day 0	Day 20	10	131±4	36·9±6·4	0	10
		Days 0 and 20	Day 40	5	146±9	52·8±5·9	0	5
2	Unfractionated insulin	Days 0, 7 and 14	Day 21	5	139±13	63·2±4·0	0	5
		Days 0, 7, 14, 21 and 28	Day 35	5	209±25	74·6±5·0	0	5

* Detailed information of the antigens and inocula is given in the text.

Guinea-pigs

All animals used in the last two experiments (Nos 3 and 4), with the exceptions of those mentioned above, remained healthy and gained weight. Blood-sugar concentrations were not measured during life but no animals showed any clear evidence of diabetic stigmata (weight loss, polyuria, polyphagia, etc.). Glucose concentrations measured in blood obtained at times of killing varied from 116 to 520 mg/100 ml but neither glucose nor ketones were ever found in urine obtained from the bladders.

As shown in Table 2, labelled insulin was bound by sera from all groups of animals except those used for controls and those immunized with homogenates of guinea-pig islets (experiment 4). Binding of labelled insulin increased as immunizations with rat islets and bovine 'A' component progressed but sera from neither of these two groups of guinea-pigs bound more labelled hormone than that from insulin-immunized animals.

Among the guinea-pigs immunized with insulin derivatives (Expt 3), pancreatic lesions were most common in those receiving the bovine 'A' component. Fifteen (38%) of these animals were found to have lesions compared with three (14%) animals immunized with 'single peak' insulin and two (9%) control animals. Among guinea-pigs immunized with 'A' component, lesions appeared more frequently in the early (50%, 30 days) than in the later stages (40%, 60 days; 10%, 90 days) of immunization. Among guinea-pigs immunized with homogenates of islet tissue (Expt 4), pancreatic lesions appeared more frequently in immunized (18/26) than in control animals (1/14). No matter which antigen was used, the induced lesions appeared to be of the same type. They consisted of very localized

TABLE 2. Labelled insulin binding by sera and pancreatic lesions of guinea-pigs immunized with bovine insulin, bovine 'A' component and homogenates of islets of Langerhans

	Immunization programme*					Necropsy findings				
						Labelled insulin binding by sera† (%/1·0 μl)			Pancreatic lesions	
Expt No.	Antigen	Injections given	Killed	Animals (*n*)	Blood-sugar (mg/100 ml)	2·0 mU	1·0 mU	0·5 mU	Present	Absent
3	Nil	Day 0	Day 30	13	117±3		1·8±0·6	2·6±1·5	1	12
		Days 0, 30 and 60	Day 90	10	135±4		0·7±0·2	0·1±0·1	1	9
	Single peak Insulin	Day 0	Day 30	14	118±5		36·0±4·3	52·0±4·6	2	12
		Days 0, 30 and 60	Day 90	8	109±18	69·6±9·1			1	7
	'A'	Day 0	Day 30	20	137±8		12·2±3·7	21·2±5·0	10	10
		Days 0 and 30	Day 60	10	172±16	12·5±4·4	17·2±5·0	28·1±6·2	4	6
	Component	Days 0, 30 & 60	Day 90	10	134±7	28·8±4·4	53·7±7·6	76·7±7·1	1	9
4	Nil Control	Nil	Day 0	6	210±32		0	012±0·2	0	6
		Days 0, 21 and 42	Days 63–65	8	167±14		0	0·3±0·2	1	7
4	Guinea-pig	Day 0	Day 21	3	264±129		0·4±0·3		2	1
		Days 0 and 21	Day 41	3	224±48		0·2±0·1		3	0
	Islets	Days 0, 21 and 42	Day 62	5	191±33		0·5±0·3		3	2
	Rat	Day 0	Day 20	3	182±39		0·3±0·2	0	1	2
		Days 0 and 21	Day 38	3	229±50		6·5±6·0	6·5±5·5	3	
	Islets	Days 0, 21 and 42	Day 56–62	9	221±22		22·6±9·3	29·8±9·6	6	3

* Detailed information on the antigens and inocula is given in the text.

† Sera (1·0 μl) were incubated with a constant amount of labelled insulin (*c* 10 μu) and the indicated amounts of unlabelled hormone (mu).

and, usually, well circumscribed areas of infiltration by small lymphocytes. Such infiltrating cells were most frequently near small pancreatic ducts and blood vessels (Fig. 2a) in the exocrine tissues. Occasionally such lesions caused obstruction and dilatation of small pancreatic ducts (Fig. 2b). Collections of small lymphocytes were sometimes seen around islets of Langerhans (Fig. 2c) but such concentration did not appear to be selective when infiltrations were extensive (Fig. 2d). No plasma cells were ever seen. Eosinophils were very occasionally found near large ducts and between acinar cells but did not appear in the areas infiltrated by lymphocytes. No correlation could be established between the incidence of these lesions and the levels of insulin-binding antibodies found in the plasma. No comparable lesions were found in other tissues (livers, kidneys, etc.) removed either from these guinea-pigs or from the rabbits.

DISCUSSION

The term 'insulitis' is currently used to indicate a lesion characterized by 'lymphocytic infiltration in and about the islands of Langerhans' of the pancreas (Warren *et al.*, 1966). LeCompte (1958) considered it rare and 'practically confined to cases (of diabetes) of recent onset in children'. Doniach & Morgan (1973) found no such lesions in thirteen young diabetics, and Maclean & Ogilvie (1959) reported only three instances among twenty-two cases, all of whom had died in the early stages of their disease.

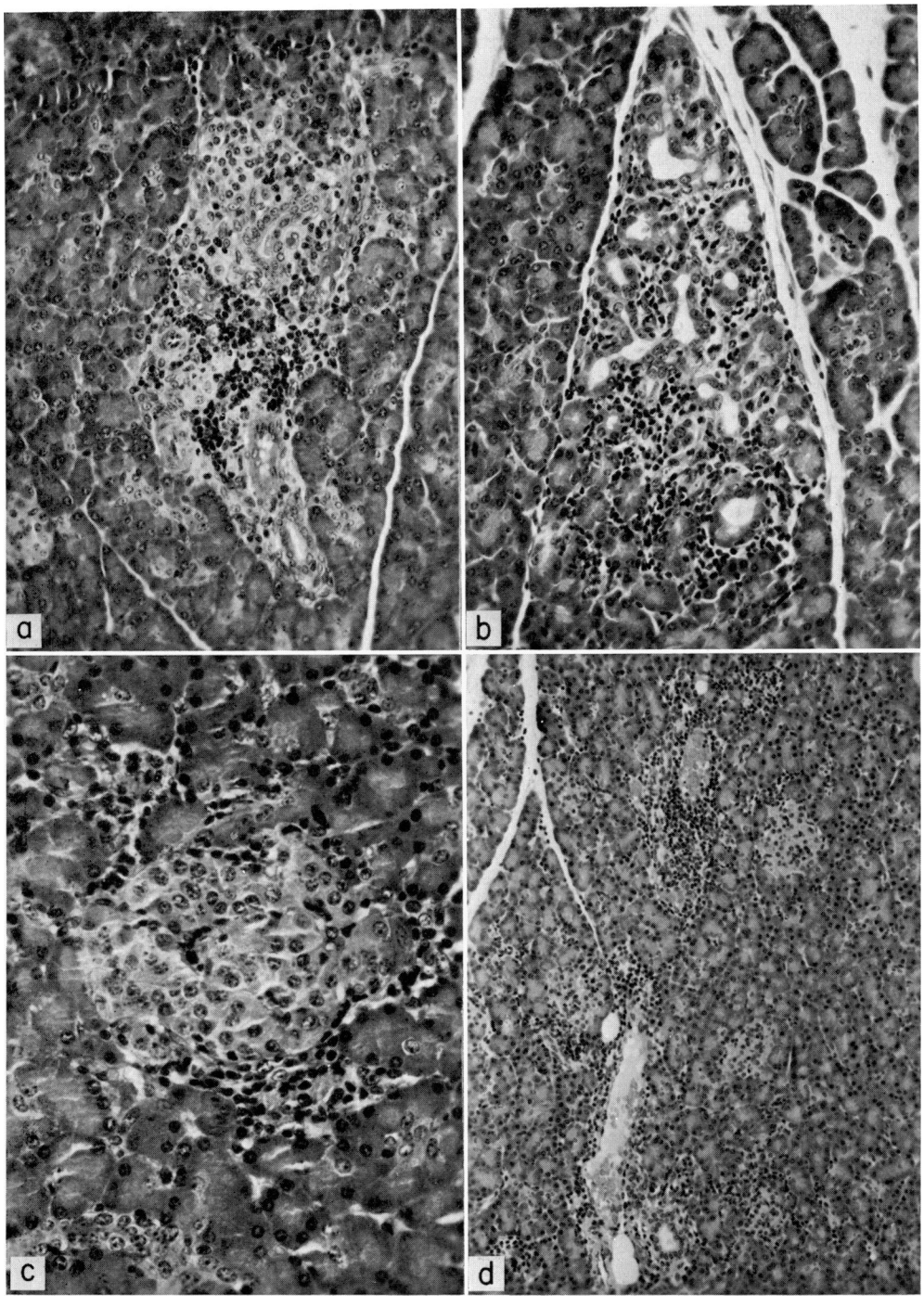

FIG. 2. Sections of pancreatic tissue from guinea-pigs killed 4–6 weeks after initial immunization with bovine 'A' component (a, b and c) or a homogenate of rat islets (d). The sections (H & E; magnification × 140–375) show lymphocytic infiltration (a) around small blood vessels and pancreatic ducts, (b) of acinar tissue with dilatation of small pancreatic ducts, (c) around a small islet of Langerhans, and (d) adjacent to small blood vessels and ducts without significant involvement of a nearby islet of Langerhans.

Gepts (1965), on the other hand, showed extensive lymphocytic infiltration of the islets in eight and lesser involvement in another seven out of a total of twenty-two young diabetics dying within 6 months of diagnosis. It is difficult to assess the real incidence of this lesion in juvenile diabetes but its appearance has suggested to some that this disease might be the result of either a viral infection or an autoimmune process. There have been numerous reports of infections preceding the onset of juvenile diabetes (Gunderson, 1927; John, 1934; Brown, 1956) and in recent years the Coxsackie-B_4 virus has been actively considered as a potential causative agent (Gamble *et al.*, 1969; Gamble & Taylor, 1969). In support of the autoimmunity hypothesis, it has been shown that circulating antibodies to gastric and thyroid antigens are more frequently found in the sera of juvenile diabetics than in those of other diabetic or non-diabetic subjects (Ungar *et al.*, 1968; Goldstein *et al.*, 1970; Irvine *et al.*, 1970; Nerup & Binder, 1973); and that juvenile diabetics also show evidence of cellular hypersensitivity to pancreatic antigens (Nerup *et al.*, 1973a; MacCuish *et al.*, 1974). Very recently, Lendrum, Walker & Gamble (1975) have also shown that sera of juvenile diabetics under the age of 17 years frequently (almost 50%) contain antibodies which react with human islet tissue *in vitro*; this very seldom occurs with sera from non-diabetic children. These observations and the early observations by Renold *et al.* (1966) that 'insulitis' could be induced in cattle by immunization with insulin have prompted several groups of investigators to attempt the induction of diabetes mellitus in experimental animals with viruses and with various pancreatic antigens.

Severe and predictable diabetic syndromes such as those which follow removal or destruction of the insulin secreting cells of the pancreas are not readily induced with viruses or by immunization with pancreatic antigens. Hyperglycaemia has been reported in mice following infection with encephalomyocarditis (Craighead & Steinke, 1971) and Coxsackie-B_4 (Coleman, Gamble & Taylor, 1973) viruses. Some of these animals do become frankly diabetic but others remain unaffected or become only transiently hyperglycaemic. Transient hyperglycaemia is also more commonly seen than permanent diabetes in rabbits immunized with porcine or bovine insulins (Toreson *et al.*, 1968; Lee *et al.*, 1969; Kloppel *et al.*, 1972, 1974). Transient intolerance to glucose or mild hyperglycaemia have been detected in mice immunized with islet tissue (Nerup *et al.*, 1974a). Finally, no such metabolic abnormalities were demonstrated in cattle immunized with insulin (LeCompte *et al.*, 1966; Renold *et al.*, 1966), in rats immunized with extracts of foetal calf pancreas (Nerup *et al.*, 1973b) or in rats given repeated injections of homologous islet homogenates (Heydinger & Lacy, 1974). In the present series of experiments with rabbits and guinea-pigs it is possible that some of the immunized animals may have suffered minor degrees of insulin deficiency during life. However, with the exceptions of the three rabbits and three guinea-pigs specifically mentioned above, all the immunized animals remained in good health and gained weight. Blood-sugar concentrations were sometimes found to be high at death or, in the cases of some rabbits, during life. Since no glucose was ever found in urine taken from the bladder of any animal at the time of death, it was concluded that high levels of blood-glucose observed sometimes at that time (Tables 1 and 2) were probably due to acute trauma and were not the result of any chronic metabolic abnormality. In other words, none of the present animals showed signs of persistent severe insulin deficiency during life and none were diabetic at the various times of death.

Reports of the lesions induced in animals by immunization with insulin or other pancreatic antigens have also varied. The infiltrating cells found in islets of cattle immunized with insulin were described by LeCompte *et al.* (1966) as lymphocytes 'of the "small" variety with round or slightly oval nuclei and scanty cytoplasm'. By contrast, Lee *et al.* (1969) reported that in insulin-immunized rabbits 'the major component of infiltrating cells was the large lymphocyte with abundant homogeneous cytoplasm'. Most investigators, however, have not described the infiltrating cells which they have seen, merely referring to them as 'lymphocytes' or 'small lymphocytes'. Such cells have been variously reported within capillaries traversing islets, in intimate contact with cells within the islets, in the immediate vicinity of islets, or in the acinar tissues around small blood vessels and pancreatic ducts. Freytag *et al.* (1973) have in fact suggested that there may be two distinct types of induced lesion which they have termed 'peri-ductulitis' and 'peri-insulitis' according to the site of lymphocyte concentration. The frequency with which lymphocytic infiltration is to be found within a given affected pancreas is seldom

reported. However, LeCompte *et al.* (1966) have reported at one extreme that in one of their insulin-immunized cattle 'practically every islet showed some alteration'. At the other, and in spite of their claim that mononuclear infiltration had been seen in many islets, Nerup *et al.* (1974a) were unable in a blind study to demonstrate any statistically significant difference between the incidences of lesions in control and immunized mice. Extensive infiltration of the islets in rabbits (Toreson *et al.*, 1968; Kloppel *et al.*, 1972, 1974), cattle (LeCompte *et al.*, 1966) and mice (Nerup *et al.*, 1974a) has been associated with degranulation of the β-cells and, in some cases, with persistent hyperglycaemia. After prolonged immunization, fibrosis has also been reported within the islets (Lee *et al.*, 1969; Heydinger & Lacy, 1974). Conspicuously absent from reported islet lesions have been signs of necrosis, a common feature of islets in mice infected with viruses (Burch *et al.*, 1971; Craighead & Steinke, 1971). In rats (Nerup *et al.*, 1973b) and mice (Nerup *et al.*, 1974a) given a single injection of antigen and in rabbits given four weekly inocula of antigen (Kloppel *et al.*, 1974), lesions found in the pancreas during the first 2–4 weeks were no longer demonstrable at a later stage. It is against this background of the very varied experiences of others that the present observations have to be discussed.

First, and contrary to the experience of Federlin (1971), pancreatic lesions were induced in guinea-pigs with a variety of pancreatic antigens. Federlin (1971) found pancreatic lesions in only two of an undefined total number of guinea-pigs which had been immunized with a recrystallized preparation of insulin; one showed mild infiltration of small islets after 8 weeks and the other was found to have more extensive lesions after 83 weeks. Of twenty-two guinea-pigs immunized in this laboratory with a similar unfractionated preparation of insulin, only two showed signs of mild islet fibrosis after 8 months and none exhibited evidence of acute cellular infiltration of the pancreas (unpublished observations). As others have suggested (see above) the acute lesions observed in the present studies might well be transient (Table 2). Those induced by bovine 'A' component, for example, were seen more frequently in animals killed after 30 days than in either of the groups killed later after 60–90 days. The acute lesions did not appear to be due to insulin itself for they appeared in animals immunized with bovine 'A' component or with homogenates of homologous or heterologous islets but not when the antigen was a preparation of insulin (single peak) from which contaminants of high molecular weight (i.e. 'A' component) had been removed. The induced lesions were not numerous, no more than four or five highly localized areas of infiltration being seen in cross-sections of four to six pieces of tissue from a single pancreas. The infiltrating cells were invariably lymphocytes of the 'small' variety and they were found most frequently round small pancreatic ducts and blood vessels. The islets themselves were seldom clearly affected and their β-cells were invariably well granulated. From these observations it seems reasonable to conclude that a form of transient periductulitis can be induced in guinea-pigs with antigens containing pancreatic constituents other than insulin, but that these lesions are not severe, are transient and do not appear to cause insulin deficiency *in vivo*.

In the second series of experiments (Table 1, experiments 1 and 2), no evidence of pancreatic lymphocytic infiltration could be found in rabbits killed 3–6 weeks after the first of one or more injections of bovine insulin or bovine 'A' component. Both Lee *et al.* (1969) and Kloppel *et al.* (1972, 1974), however, found signs of transient or permanent diabetes and of lymphocytic infiltration of the pancreatic islets in a high proportion of the rabbits which they had immunized at weekly intervals with unfractionated crystalline bovine insulin in Freund's complete adjuvant. Failure to induce such effects in the first experiment of the present study (experiment 1) could have been due to the use of a different adjuvant, *H. pertussis* vaccine instead of killed *M. butyricum*. However, when small groups of rabbits were immunized with unfractionated bovine insulin in the traditional Freund's Adjuvant at weekly intervals (Expt 2) the results were the same. It does not therefore seem that any rational explanation for the discrepancy is yet possible.

Finally it should be emphasized that there seems to be no consistency in severity, frequency or distribution of the pancreatic lymphocytic infiltrations which have been induced by different investigators. This could be due to differences between the antigens used, to differences between the programmes of immunization, or to differences in susceptibility of the various species studied. There remains the possibility, however, that genetic factors could play a role. Craighead & Higgins (1974),

for example, have found that the diabetogenic effect of one strain of virus can be influenced by the strain of mouse used for study, and that resistance to this effect can be transmitted hereditarily. Again, Nerup *et al.* (1974b) have shown that HL-A antigens are more frequently found in diabetic than in non-diabetic human subjects and that the increase among diabetics is almost entirely due to the insulin-requiring diabetics. These observations suggest that the susceptibilities of experimental animals to the diabetogenic effects of pancreatic immunogens could be genetically determined, and that more appropriate species than those currently used should be sought.

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