PROCEEDINGS OF THE NUTRITION SOCIETY
A Scientific Meeting was held at the University of York on 4–6 July 1990

Symposium on 'Health implications of nutrient-gene interactions'

Possible dietary factors in the induction of diabetes and its inheritance in man, with studies in mice

BY JOHN M. STOWERS AND STANLEY B. W. EWEN
Department of Pathology, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZY

My interest in this general subject started in 1981 when I had a telephone call from Iceland from a doctor there who had worked with me in the Aberdeen Diabetic Clinic for 1·5 years. Since returning to Iceland, where he was responsible for the Diabetic Service he had completed a meticulous survey of all the insulin-dependent patients in the population of just under 0·25 million people. On analysing the data he came upon an unexpected and highly significant finding. This was that a great preponderance of the boys who developed diabetes before their fifteenth birthday were born in the month of October. The statistical significance of this finding turned out to be $P<0{\cdot}00001$ (Helgason & Jonasson, 1981; Fig. 1).

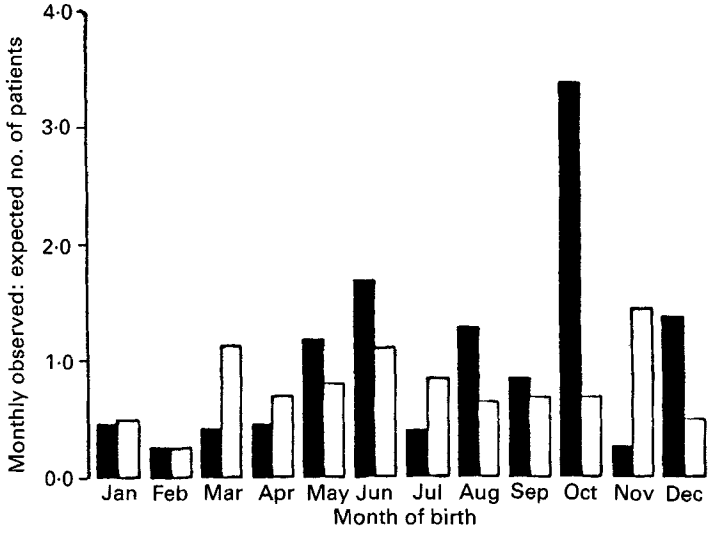

Fig. 1. Seasonal distribution in month of birth of ketosis-prone diabetics in Iceland aged 0–14 years at diagnosis, (■), males (*n* 56); (□), females (*n* 41). The seasonal distribution is adjusted for the yearly average per month of all births (*n* 65·786) in the total population 1964–1978. The observed no. of boys born in October was sixteen (cf. expected no. of 4·73). The difference between the no. of boys born in October and those born in other months was significant ($P<0{\cdot}00001$) (Helgason & Jonasson, 1981).

Dr Helgason merely told me on the phone that he had a most interesting new piece of information about diabetes and he wished to come to Aberdeen soon to discuss it. This he did. We realized that the implication was that something happening in one or both parents at about the time of conception was affecting the future risk of diabetes in their sons. The October-born boys, delivered at full term, must have been conceived towards the end of December or in January, so we had to consider any special factor which could have affected the parents at this time. I suspected that some special liquid refreshment at the time of Christmas and the New Year could have played a part, but Dr Helgason thought that this was not likely, and indeed his studies showed that the parents of the October-born diabetic boys took rather less alcohol than those of non-diabetic boys born at that time. Tobacco is another mutagen but again it was smoked by a smaller, rather than a larger percentage of the parents of the October-born diabetic boys. As I had no more ideas to contribute I went to bed in the small hours, while Dr Helgason paced up and down the passage deep in thought. At breakfast next morning he was unexpectedly cheerful and made the perceptive suggestion that a national delicacy, Icelandic smoked cured mutton (ISCM), might carry the diabetogenic agent for at least one-quarter of the annual production is consumed during the 10 d period of Yule. Helgason knew that the process of preserving the mutton and preventing botulism could lead to the formation of N-nitroso compounds and that such compounds can be mutagenic. In fact they are recognized as one of the causes of cancer of the stomach in Iceland. Furthermore, Gough *et al.* (1978) have shown that in the UK over 82% of the total nitrosamines in a mixed diet come from the cured-meat component.

Commercial curing of the mutton was introduced in Iceland in 1940 and before that a previous survey had shown that the incidence of insulin-dependent diabetes was very low (Albertsson, 1953). Helgason knew that he would have to get the currently available smoked cured mutton analysed for N-nitroso compounds before he could confirm and publish his theory. Few laboratories will undertake such work because of the carcinogenicity of the nitrosamines, but five specimens of ISCM were accepted by the Thermo-Electron Corporation in Massachusetts for analysis of the volatile nitrosamines at considerable expense. Variable but sometimes relatively high concentrations of these N-nitroso compounds were found. These results and those of Helgason's epidemiological survey were published in the *Lancet* (Helgason & Jonasson, 1981).

As Helgason had no facilities for work on experimental animals in Iceland, he asked if I could suggest anyone suitable in Aberdeen. I was fortunate to know a young pathologist interested in the endocrine system and he is Stanley Ewen, my co-author. We chose a strain of mice which was not prone to spontaneous diabetes, that is, CD1 Swiss albino mice obtained from the Charles River Company. Samples of ISCM were cooked in exactly the same way as in Iceland and after mincing were fed to the mice on alternate days starting 10 d before mating. This test diet was continued during the 3 weeks of pregnancy and was also provided for the progeny after they were weaned in a further 3 weeks. Starting from 4 weeks of age the plasma glucose of the progeny was measured in capillary specimens taken from the retroorbital sinus under deep sodium amytal sedation 90 min after a standard glucose load had been given intraperitoneally. The glucose was given in a dose of 2 mg/g body-weight and dissolved in 154 mM-saline (9 g sodium chloride/l). The animals were considered to be diabetic if values exceeded the mean for the controls by more than 3·7 SD. In the experimental group thirty of 184 males, that is 16·3%, and five of 118 females, that is 4·2%, became diabetic. No abnormal

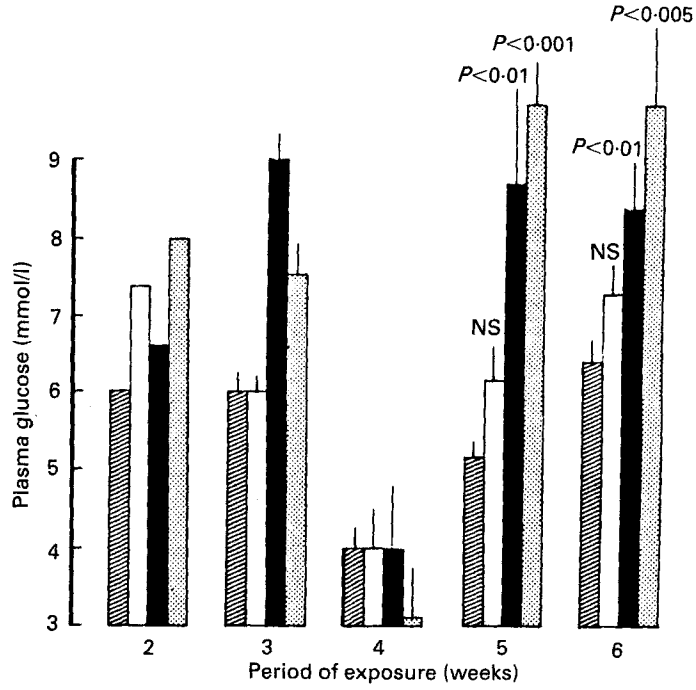

Fig. 2. Mean plasma glucose values of male progeny of mice variously exposed to Icelandic smoked cured mutton (ISCM). (▨), Control (no ISCM); (□), father; (■), mother; (▦), both were previously fed on ISCM. At 2 and 3 weeks, values represent mean non-fasting, plasma glucose values. At 4–6 weeks, the values represent mean single point glucose tolerance tests. Mean values for experimental mice were significantly different from those of controls (2-tailed test). NS, not significant. Values are means with their standard errors represented by vertical bars (from Helgason *et al.* 1982).

results were found in the parent mice or in the ninety-six control progeny (fifty-three males, forty-three females). In four diabetic mice the plasma insulin at 90 min ranged from 9–20 μU/ml, compared with 25–45 μU/ml in eight control mice ($P<0·005$).

On histological examination the islets of Langerhans tended to be larger in the diabetic male offspring of the parent mice fed on ISCM than those of the corresponding control mice. Many of the beta cells were condensed with crenated irregular nuclear membranes and strongly eosinophilic cytoplasm. There was only a little lymphocytic infiltration and there were virtually no mitoses, except in the alpha-cell region (Helgason *et al.* 1982).

In the second group of experiments a further effort was made to imitate more closely the exposure to ISCM suggested as the explanation of the increase for the number of Icelandic diabetic boys born in the month of October. Tests were done on four separate groups of CD1 mice (Fig. 2): 1, controls not exposed to ISCM; 2, the father only had ISCM; 3, the mother only had ISCM; 4, both parents had ISCM.

In all the test groups the feeding of ISCM stopped before mating occurred and it was not resumed during the pregnancy or given to the weanlings. The differences in the 90 min plasma glucose values were relatively small in the four groups of progeny up to about 4 weeks of age (Fig. 2), but at 5 and 6 weeks there was a significant increase in those of

https://doi.org/10.1079/PNS19910038 Published online by Cambridge University Press

the male progeny when both parents or only the mother had been fed on the ISCM. No such increase was seen in the plasma glucose values of the female progeny. There were contrasting results at 4 weeks of age when the male progeny had abnormally low plasma glucose values, especially when both parents had been fed on ISCM. Only about half these mice survived the test. Their hypoglycaemia may have been due to the release of endogenous insulin from their damaged beta cells. This would stimulate the early effects of diabetogenic doses of alloxan given to experimental animals. This second group of experiments showed that ISCM could induce hyperglycaemia and actual diabetes in the male progeny of CD1 mice, even although the feeding of the meat ceased before fertilization. The diabetes was, however, not insulin-dependent.

At this point I took over most of the laboratory work since I had retired. An essential collaborator has been, and still is, Dr J. R. A. Pollock, a Scientific Consultant in Reading, specializing in assessing contaminants in food and drink. He had measured both the volatile, and the less studied involatile, N-nitroso compounds in our specimens of ISCM. With his help we then tried to fractionate the smoked cured meats to see which fractions carried the factor or factors inducing diabetes in the F1 generation. At this time I decided to measure the fasting as well as the 90 min plasma glucose on the Beckman analyser and this meant changing the way the mouse blood was obtained. This is because the sodium amytal narcosis used for the retroorbital sinus approach greatly slows the speed of absorption of glucose from the peritoneal cavity and so could not be used for the fasting specimen if a meaningful 90 min specimen was to be obtained. Instead we used the tail tip and an anaesthetic spray, which does not affect the blood glucose level. For this intraperitoneal glucose tolerance test (IPGTT), CD1 mice at least 6 weeks old (20–30 g) were used and were fasted for 18 h but with free access to water. Blood from the tail tip was collected in heparinized capillary tubes and transferred to capped containers with 0·1 mg sodium fluoride to block glucose uptake by the leucocytes. Plasma glucose was measured on a Beckman analyser at time 0 and at 90 min after an intraperitoneal injection of 2 mg glucose/g body wt as a 200 g/l solution in 154 mmol NaCl.

As in most of the studies the fasting blood samples were normal, only the 90 min samples are considered. Mean 90 min plasma glucose levels (mmol/l) were: males 6·5 (SD 1·81); females 5·7 (SD 1·09), based on thirty-one male and thirty-two female normal CD1 mice. Diabetes defined as at least 3 SD above mean; that is, for males more than 12·0 mmol/l and for females more than 9·5 mmol/l.

The British mutton and smoked cured mutton were divided into six fractions and there were in addition four fractions containing the known diabetogenic nitrosamine, nitrosothiazolidine carboxylic acid (NTCA) in concentrations varying from 0–3·5 μg/g. These ten fractions were reconstituted in five different samples of meat so that each contained four fractions in the same concentrations in which they originally occurred in the meat (Table 1). The fractions not shown to be diabetogenic are listed in Table 2 with details of the mean 90 min plasma glucose values for each and the numbers of tests used. The fractions which were consistently diabetogenic are similarly listed in Table 3. These three fractions clearly differ markedly from the other five in their diabetogenicity. A preliminary conclusion may be drawn that the smoking and curing seems to exert its diabetogenic effect only in the fat fraction, but there was also some diabetogenic effect from the aqueous extracts of fresh mutton. The diabetogenicity of NTCA was confirmed in concentrations of at least 3·5 μg/g.

Table 1. *Fractions of British smoked cured and unsmoked mutton*

1. Water-extracted, largely fat free
   (a) Smoked cured
   (b) Unsmoked, uncured
2. Water extract of meat concentrated and re-added to meat in original proportions
   (a) Smoked cured
   (b) Unsmoked, uncured
3. Fat
   (a) Smoked cured
   (b) Unsmoked, uncured
4. Nitrosothiazolidine carboxylic acid

One fraction from fractions 1–4 was present in each of five samples of meat.

Table 2. *Fractions of meat found not to be diabetogenic without the coexistence of the three diabetogenic ones in male progeny of CD1 mice*

(Mean values for no. of determinations shown in parentheses)

Type of meat	Proportion diabetic	Mean 90 min plasma glucose (mmol/l)
Water-extracted meat		
(a) Smoked cured	0/16	5·6 (32)
(b) Unsmoked, uncured	0/4	7·9 (12)
Water-extracted meat, smoked cured	0/20	5·9 (46)

Table 3. *Fractions of meat consistently diabetogenic in male progeny of CD1 mice*

(Mean values for no. of determinations shown in parentheses)

Type of meat	Proportion diabetic	Mean 90 min plasma glucose (mmol/l)
Water extract of meat unsmoked, uncured	3/9	10·5 (60)
Fat, smoked cured	5/11	11·6 (22)
NTCA >3·5 μg/g	2/2	12·7 (4)

NTCA, nitrosothiazolidine carboxylic acid.

TRANSMISSION OF DIABETES IN SUCCESSIVE LITTERS

In some of the fractionated meat studies, the parent mice, which had eaten the diabetogenic meat for 10 d, were allowed to have several litters. This permitted us to see if the diabetogenic effect seen in the first litter persisted in subsequent litters. Both male and female progeny were tested but only the males showed any significant rise in plasma glucose. The results are shown in Table 4.

Although none of the eleven male progeny in the second litter was found to be diabetic, four of twelve in the third litter developed diabetes. Thus, the nitrosamine-

https://doi.org/10.1079/PNS19910038 Published online by Cambridge University Press

Table 4. *Incidence of diabetes in male progeny in successive litters of CD1 mice fed on nitrosamine-tainted meat before mating for first litter*

(Mean values for no. of determinations shown in parentheses)

Litter no.	No. of mice	Mean 90 min plasma glucose (mmol/l)	Proportion diabetic
1	8	11·6 (12)	3/8
2	11	8·4 (15)	0/11
3	12	9·6 (22)	4/12

Table 5. *Effect of subdiabetogenic intraperitoneal streptozotocin (STZ) to one or both parent CD1 mice on the glucose tolerance of their male progeny*

	STZ	Mean 90 min plasma glucose (mmol/l)	*n*	No. diabetic
Father	+	13·0	6	3
Mother	+			
Father	○	11·6	22	5
Mother	+			
Father	+	10·2	3	1
Mother	○			

+, STZ-treated; ○, untreated.

tainted meat, which did not produce diabetes in the parent mice which ate it, continued to produce diabetes for several months in subsequent litters.

SUBDIABETOGENIC DOSES OF STREPTOZOTOCIN (STZ)

In the next set of experiments the mouse IPGTT was used to study the effect of repeated small doses of the well-known N-nitroso diabetogenic agent, STZ, not only in the recipient mice but also in the F1 and F2 generations. STZ is a methylnitrosourea derivative of deoxyglucose and the glucose moiety of the molecule makes it about ten times as diabetic as the parent methylnitrosourea, probably because the glucose carries the toxicity selectively to the glucose receptors in the beta cells of the islets of Langerhans. Sandy Gordon, then a medical student working with Dr Ewen in 1982, had shown that repeated subdiabetogenic doses of STZ could induce diabetes in the male progeny, sparing the parents. I tried to repeat the study and to see if the diabetes in the progeny was hereditable.

The STZ was made up freshly each day in citrate buffer pH 4·5 for each of the seven consecutive daily intraperitoneal injections. The daily dose was 1·7 mg/kg body-weight, that is a total of 11·9 mg/kg for the 7 d course. For STZ to be diabetogenic to the recipient in a single dose about 200 mg/kg is needed. None of the six female mice showed any significant effect on their glucose tolerance, but two of the three males became diabetic with a mean 90 min plasma glucose level of 12·0 mmol/l. These male and female mice were then mated with each other and with normal CD1 mice. The glucose tolerance of their male offspring is shown in Table 5. There was a gap of 30 d between the end of

Table 6. *Intraperitoneal glucose tolerance test results in male F2 generation of CD1 grandparents given seven subdiabetogenic doses of intraperitoneal streptozotocin (STZ)*

No. of STZ grandparents	No. of F2 males	No. diabetic	Mean plasma glucose (mmol/l)
2	8	2	8·9
3	15	3	10·0

the course of STZ to one or both parents and the time of mating, so that there was no question of direct transmission of the STZ to the fetuses. An alternative explanation is that STZ in subdiabetogenic doses for the mother produced highly specific changes in the germ cells which lead to diabetes in the male progeny.

The results with the male F2 generation are shown in Table 6. None of the female F2 generation was found to be diabetic 24 and 133 d after the end of the course of STZ.

STUDIES ON GROUND-UP BETEL (*ARECA CATECHU* L.) NUT IN THE DIET

When Dr Barbara Boucher of the London Hospital read Dr Helgason's original paper (Helgason & Jonasson, 1981) and the later one from Aberdeen in the *Lancet* (Helgason *et al.* 1982) about the possible diabetogenic effect of nitrosamines in smoked cured mutton she wondered whether sources of dietary nitrosamine could be acting in a similar manner in the population she served in the local diabetic clinic. She works in East London where the incidence of non-insulin-dependent diabetes in the large immigrant population from the Indian subcontinent is four to five times higher than that in the native white Londoners (Mather & Keen, 1985), so dietary differences between these two groups were considered. Ramachandran *et al.* (1988) have shown that Indians have a similarly high prevalence of diabetes in their own country. The Asian immigrants have brought with them their traditional way of life and this includes the habit of chewing betel nuts made up into so-called quids with betel leaves, slaked lime, catechu and often tobacco (Ghosh & Ghosh, 1988). The betel nut contains arecoline which is a stimulant to the central nervous system and can be nitrosated to several strong mutagens in vivo (Wenke *et al.* 1984), the whole mixture being carcinogenic to the mouth and upper alimentary tract. The mutagenic effect of betel leaf extract has been shown, for instance, in its ability to increase chromatid exchanges in lymphocyte cultures (Sadasivan *et al.* 1978). Betel or Areca nuts, like tobacco, are also potent sources and precursors of nitrosamines. In view of these facts Dr Boucher and the present authors are co-operating in a study in which the clinical part has started at the London Hospital and the animal work and histology are being done in Aberdeen. We are also enlisting help from geneticists. Dr James Pollock has confirmed that betel nuts bought in East London contain nitrosamines and he has extracted them from a small batch, so that the Areca ground-up powder could be tested on CD1 mice after extraction of nitrosamines, and also with the nitrosamines restored in another small batch at a concentration of 30 μg/g. When the nitrosamine-extracted Areca diet was fed for just 2 d to two male CD1 mice and measurements made after a further 15 d, there was a slight rise in the fasting plasma glucose (from 5·0 to 6·9 mmol/l) and a very significant rise in the 90 min value (from 6·7 to 15·4 mmol/l); both animals being diabetic. The small quantity of the extracted Areca

Table 7. *Effect of Areca (300 g/kg diet) fed for 6 d, alternating with low-nitrosamine standard RM1 diet on development of diabetes in CD1 mice*

(Mean values for two mice)

Dietary treatment	90 min plasma glucose (mmol/l)
Before Areca feeding	
♂	8·1
♀	6·2
12 d after Areca feeding for 6 d alternating with normal diet	
♂	11·2 (8·7,13·7*)
♀	9·0 (3·8–17·5*)
19 d after Areca feeding	
♂	11·0 (9·9,12·1*)
♀	8·0 (4·0–15·1*)

* Diabetic.

Table 8. *The effect of prolonged feeding of Areca diet for 14 d alternating with normal diet on intraperitoneal glucose tolerance test in CD1 mice*

(Mean values and ranges for no. of mice shown in parentheses)

	Intervals from end of Areca diet (d)...	90 min plasma glucose (mmol/l)			
		−33	+14	+21	+30
♂	(5): Mean	5·2	9·4*	7·5	7·2
	Range	3·0–6·4	7·1–11·7	2·8–11·2	5·1–9·1
♀	(3): Mean	4·9	11·5	5·0	3·9
	Range	3·8–6·4	5·1–24·0†	4·0–6·6	2·2–4·5

* Mean value was significantly different from that at −33 d ($P<0·01$).
† Diabetic.

nut with nitrosamines re-added could unfortunately be tested on only one male mouse and this was done three times starting 22 d after the 4 d period of test feeding Areca + 30 μg Areca nitrosamines/g, alternating with the normal diet. On two of these three occasions the 90 min plasma glucose (mmol/l) was in the diabetic range (12·4 and 12·9) and on the third it was slightly below it (10·2 (mean fasting value (mmol/l)) before test period 4·1, at 22 d 5·6; mean 90 min value (mmol/l) before test period 8·6, at 22 d 11·8). The suggestion from these very preliminary experiments is that not all the directly diabetogenic effect of the betel nut powder is due to the readily extractable nitrosamines.

The next experiment used 300 g ground Areca/kg made into pellets and fed for 6 d alternating with the standard RM1 diet which is chosen as being low in nitrosamines. The results are shown in Table 7. Diabetes developed in one of the two males by 12 d after the end of the Areca diet and was still present at 19 d. What was more significant was the development of a more marked diabetes in one of the four females, both at 12 and 19 d.

A more prolonged feeding of the Areca diet occurred in the next group of CD1 mice. It was fed for 14 d alternating with diet RM1 and the results are shown in Table 8. In this

study one female developed a 90 min plasma glucose as high as 24·0 mmol/l at 12 d after stopping the Areca diet, but it had resolved at 21 and 30 d. For the five males there was a significant rise ($P<0·01$) in the 90 min plasma glucose at 12 d but a return towards the pre-Areca feeding level at 21 and 30 d.

PROGENY OF PARENTS FED ON THE ARECA DIET

So far the opportunity to test the progeny of Areca-fed diabetic mice has been taken on two occasions. The male progeny of parents fed on the Areca diet for 4 d all had diabetes in two tests done 12 d apart (mean fasting plasma glucose 6·3 mmol/l, mean 90 min plasma glucose 15·9 mmol/l; *n* 3). None of the four female progeny developed diabetes. In the second study six male and three female progeny of Areca-fed diabetic mice were tested at 7 weeks of age when they were fed on Areca for 6 d. Again the females were normal but at 7 weeks one of the six males was markedly diabetic (90 min plasma glucose 9·3 (range 4·4–21·9) mmol/l) and at 10 weeks two of the six males were diabetic (90 min plasma glucose 10·5 (range 8·1–12·5) mmol/l). One of the male mice born of parents who both developed diabetes after Areca feeding and itself shown to be diabetic at 10 weeks old was killed at 36·5 weeks of age. The histology of its pancreas was very interesting, showing lymphocytic infiltration of the islets of Langerhans, similar to that seen in fatal cases of diabetes in children.

At this stage the Areca studies in mice have shown that it is, like the nitrosamine-tainted meat, able to induce diabetes in the F1 generation and is indeed more potent since some of the recipient animals have also developed diabetes. This occurred only once in the tainted meat experiments.

DISCUSSION

A very curious finding has been that the diabetes caused by the Areca nut and also by small doses of STZ can also induce diabetes in the F1 generation with beta-cell damage by an effect on germ cells, whereas larger doses can also produce diabetes in the adult by a direct action on the pancreatic islets. In one case a very specific chromosome defect in the germ cells seems to be responsible, since the diabetogenic agent affects the progeny many days after the end of the parents' exposure to it, and in the other the action appears to be mainly on the beta cells. We feel that this phenomenon can best be studied by the technique of DNA finger-printing for which we hope to have support.

In some instances the type of diabetes in the recipient animal seems to be related to the dose of the diabetogenic agent. This is true for STZ and also for the rodenticide Vacor (Lee *et al.* 1977; Karam *et al.* 1980). Smaller doses of STZ can produce diabetes in rodents with lymphocytic infiltration of the islets of Langerhans (Like & Rossini, 1976) and systemic MHC expression (Cockfield *et al.* 1989), as occurs in juvenile human diabetes, whereas larger doses destroy the insulin-producing cells without any auto-immune component in the process. In the 1960s Okamoto (1965) showed that diabetes induced in rats, rabbits and guinea-pigs by another unrelated mutagen, alloxan, with subsequent inbreeding of the diabetic animals induced spontaneous diabetes in the F5–F7 generations. He attributed this to the high blood sugar of the parents, for he could inhibit the diabetes in the next generation by correcting the hyperglycaemia in the parent

Table 9. *Sources of N-nitroso compounds*

Exogenous (intake)		Endogenous (in vivo formation)	
Lifestyle	Occupation	Intake of precursors	Formation of precursors
Food	Cutting oils	Nitrates	Nitrite from nitrate (saliva
Tobacco	Hydraulic fluid	Nitrosatable N compounds	and gastric juice)
Drugs	Rubber industry		Nitrite formation in gut
Cosmetics	Pesticide production		
Bottle teats	Detergent production		

Table 10. *Nitrosamines (μg/kg) in smoked foods (from Helgason* et al. *1984)*

	n	NT	NTCA
Smoked ham	12	0–5	0–2100
Ham paste	5	0–10	68–4400
Meat drink	3	0	10–900
Smoked sausage	12	0–5	5·5–944
Smoked tongue	1	0.4	43
Smoked salmon	3	<1	<1
Smoked oyster	1	109	167

NT, nitrosothiazolidine; NTCA, nitrosothiazolidine carboxylic acid.

for at least 4 d before fertilization took place. These results differ basically from our own because his parent animals had to be diabetic for future generations to develop diabetes.

The special susceptibility of males, both murine and human, to the diabetogenic effect of the N-nitroso compounds deserves comment. Such a male preponderance is seen also when small doses of STZ are given to laboratory animals and has been shown to be inhibited by oestrogens and increased by androgens, such as testosterone (Kromann *et al.* 1982; Paik *et al.* 1982).

A study by my co-author and Dr James Pollock has shown that a known diabetogenic nitrosamine, N-nitrosothiazolidine carboxylic acid tends to be concentrated in the gonads of mice (Helgason *et al.* 1984), so the opportunity may arise there for specific mutagenic effect on germ cells. Our evidence has suggested that the effect of producing diabetes in the next generation is more potent in the ovary than in the testes, but the paternal effect can be seen. This may seem less surprising to us now when evidence has recently been released that fathers exposed to excess nuclear radiation in their work have children with a significantly increased risk of developing leukaemia (Gardner *et al.* 1990).

Table 9 summarizes the many potential sources of N-nitroso compounds and, with regard to food, the increasing use of nitrates as fertilizers has increased their uptake into vegetables. Such nitrates can be reduced by bacteria to nitrates and form nitrosamines in the course of digestion.

Table 10 shows the content of nitrosothiazolidine and its carboxylic acid in various foods. Smoked ham, ham paste and smoked sausage have much the largest concentrations of NTCA.

Much work has been done on the carcinogenicity of numerous N-nitroso compounds and relatively much less on their diabetogenicity to the recipient or the progeny. Apart

from the nitrosamide, STZ, N-nitrosomethylurea (Wilander & Tjalve, 1975) and N-nitrosoethylurea (Anderson *et al.* 1975) have been proved to be directly diabetogenic and more recently the rodenticide Vacor, which is chemically related to the N-nitrosureas, has been shown to be diabetogenic in man (Karam *et al.* 1980). This list has been extended to nitrosothiazolidine and its carboxylic acid (Helgason *et al.* 1984).

Our studies in Aberdeen were stimulated by the finding in Iceland of the marked male preponderance of diabetes in boys born in the month of October. Our studies in mice have supported a causative role for the nitrosamines present in smoked cured mutton, traditionally eaten in Iceland at Yule time, but we make no claim that the nitrosamines are more than a single factor in a disease which seems to be caused by a cumulation of several factors in the same individual (Toniolo *et al.* 1980). These include the susceptibility to diabetes associated with certain histocompatibility complexes, which is consistent with the Icelandic findings, an autoimmune destructive process in the pancreatic islets, is shown to occur in mice subjected to repeated subdiabetogenic doses of STZ (Like & Rossini, 1976), viruses and environmental factors, such as the quantity of food and its chemical constituents (Craighead, 1978). It is in this last category that we place the N-nitroso compounds and they may well be important in foods and traditional behaviours not yet recognized. This theory has been supported in a recent paper by Dahlquist *et al.* (1990) in their thorough survey of dietary factors in relation to the increasing risk of developing juvenile-onset, insulin-dependent diabetes in Sweden.

This work owes its origin to the research and motivation of Dr Thorir Helgason of Reykjavik.

The mice for the earlier studies were provided by a grant from the Grampian Health Board and additional support at this stage came from the Icelandic Science Foundation and the Science Fund, Landspitalinn University Hospital, Reykjavik. More recently the Diabetic Clinic Research Fund of Aberdeen Royal Infirmary has paid for the mice.

Dr J. R. A. Pollock, Ladbroke Close, Woodley, Reading RG5 4DX, has provided the help we needed in the analysis and the preparation of diets containing nitrosamines.

The authors are grateful for the support of the staff of the Animal House and of the Medical Illustration Department of Aberdeen University Medical School and to Mrs Sandra Anderson who prepared the typed script.

REFERENCES

Anderson, T., McMenamin, M. & Schein, R. S. (1975). Diabetogenic activity of deoxy-2-(ethylnitrosoamino-carbonylamino)-D-glucopyranose. *Biochemical Pharmacology* **24,** 746–747.

Albertsson, W. (1953). Diabetes in Iceland. *Diabetes* **2,** 184–186.

Cockfield, S. M., Rammassar, V., Urmson, J. & Halloran, F. (1989). Multiple low dose streptozotocin induces systemic MHC expression in mice triggering T cells to release IFN-Y^1. *Journal of Immunology* **142,** 1120–1128.

Craighead, J. E. (1978). Current views on the etiology of insulin-dependent diabetes. *New England Journal of Medicine* **299,** 1439–1445.

Dahlquist, G. G., Blom, L. G., Persson, L. A., Sandstrom, A. I. M. & Wall, S. G. I. (1990). Dietary factors and the risk of developing insulin dependent diabetes in childhood. *British Medical Journal* **300,** 1302–1306.

Gardner, M. J., Snee, M. P., Hall, A. J., Powell, C. A., Downes, S. & Terrell, J. D. (1990). Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *British Medical Journal* **300,** 423–429.

Ghosh, P. K. & Ghosh, R. (1988). Effect of Betel chewing on frequency of sister chromatid exchanges in pregnant women and women using oral contraceptives. *Cancer Genetics and Cytogenetics* **32,** 211–215.

Gough, T. A., Webb, K. S. & Coleman, R. F. (1978). Estimate of volatile nitrosamine content of U.K. food. *Nature* **272,** 161–163.

Helgason, T., Ewen, S. W. B., Jaffray, B., Stowers, J. M., Outram, J. R. & Pollock, J. R. A. (1984). N-nitrosamines in smoked meats and their relation to diabetes. *IARC Scientific Publications* **57,** 911–920.

Helgason, T., Ewen, S. W. B., Ross, I. S. & Stowers, J. M. (1982). Diabetes produced in mice by smoked/cured mutton. *Lancet* **ii,** 1017–1022.

Helgason, T. & Jonasson, M. R. (1981). Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* **ii,** 716–720.

Karam, J. H., Lewitt, C. W., Nowlan, R. E., Frankel, B., Fijiya, H., Freedman, Z. R. & Grodsky, G. M. (1980). Insulinopenic diabetes after rodenticide (Vacor) ingestion. *Diabetes* **29,** 971–978.

Kromann, H., Christy, M., Lernmark, A., Nedergaard, M. & Nerup, J. (1982). The low dose streptozotocin murine model of type I (insulin-dependent) diabetes mellitus. Studies in vivo and in vitro of the modulating effect of sex hormones. *Diabetologia* **22,** 194–198.

Lee, T. H., Kang, J. C., Han, M. D., Kim, J. S., Roh, J. W., Chung, M. Y. & Choi, B. J. (1977). A clinical analysis of rodenticide 'RH 787' intoxination in Korea. *Journal of Korean Diabetic Association* **4,** 43–51.

Like, A. A. & Rossini, A. A. (1976). Streptozotocin-induced pancreatic insulitis: New model of diabetes mellitus. *Science* **193,** 415–417.

Mather, H. M. & Keen, H. (1985). The Southall Diabetes Survey: prevalence of diabetes in Asians and Europeans. *British Medical Journal* **291,** 1081–1084.

Okamoto, K. (1965). Apparent transmission of factors to offspring by animals with experimental diabetes. In *On the Nature and Treatment of Diabetes,* pp. 627–640 [B. S. Liebell and G. A. Wrenshall, editors]. Amsterdam: Excerpta Medica.

Paik, S. G., Michelis, M. A., Kim, Y. T. & Shin, S. (1982). Induction of insulin-dependent diabetes by streptozotocin. Inhibition by estrogens and potentiation by androgens. *Diabetes* **31,** 724–729.

Ramachandran, A., Jali, M. V., Mohan, V., Svehalath, C. & Viswanathan, M. (1988). High prevalence of diabetes in an urban population in South India. *British Medical Journal* **197,** 587–590.

Sadasivan, G., Gulab, R. & Kumari, C. K. (1978). Chromosome damaging effect of betel leaf. *Mutation Research* **57,** 183–185.

Toniolo, A., Anodera, T., Yoon, J. W. & Notkins, A. L. (1980). Induction of diabetes by cumulative environmental insults from viruses and chemicals. *Nature* **288,** 383–385.

Wenke, G., Rivenson, A., Brunnemann, K. D., Hoffmann, D. & Bhide, S. V. (1984). A study of betel quid carcinogenesis. II formation of N-nitrosamines during betel quid chewing. *IARC Scientific Publications* **57,** 859–866.

Wilander, E. & Tjälve, H. (1975). Uptake of labelled n-nitrosomethylurea in the pancreatic islets. *Virchows Archiv* **367,** 27–33.

Printed in Great Britain