control of hyperglycaemia at least at the end of the night instead of running the risk of severe nocturnal hypoglycaemia [10]. Of course 'pragmatic' studies [3] aimed at choosing the most practical treatment for achieving near-normalization of blood glucose levels in diabetic patients who need euglycaemia cannot readily be extrapolated since the treatments studied must be given under routine conditions. Pragmatic trials studying pump treatments are certainly needed. The 'feasibility phase' of the National Institutes of Health trial will thus probably be useful. But, as far as trials on insulin treatment are concerned, we would like to suggest that it would be more rational to spend funds not on explanatory trials but on methods designed to simplify the normalization of glycaemia and to make it safe and available for the majority of diabetic patients.

Yours sincerely
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Glucagon Induced Hyperkalaemia in Diabetes

Dear Sir,

Van Gaal and de Leeuw recently reported in a Letter to the Editor [1] their data showing a highly significant positive correlation between potassium and high blood glucose levels (> 20 mmol/l) in 22 admissions for diabetic ketoacidosis. They concluded that 'hyperglycaemia is the single most important cause of hyperkalaemia in these patients'; without evaluating any other factors.

We recently documented a hyperkalaemic role of glucagon in normal subjects [2]. Van Gaal and de Leeuw in their Letter suggest that we concluded from our work in normal volunteers that hyperglucagonaemia is a major cause of hyperkalaemia in decompensated diabetes. In fact we reported that circulating glucagon might be a contributory factor in the change in potassium homeostasis in decompensated diabetes. In further studies, we documented that in insulin-dependent diabetic patients the increase in potassium following the withdrawal of an insulin infusion, which had maintained the patients euglycaemic overnight, was not correlated with the increase in blood glucose, while it was abolished by somatostatin (which inhibits glucagon) [3]. These data do not exclude the importance of other mechanisms, but suggest again that the increase in glucagon which occurs during non-ketoacidotic decompensation could at least play a part in causing hyperkalaemia. This latter phenomenon could be due to mobilization of potassium from the liver as we have documented in normal subjects [4].

Yours sincerely,
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Virus-Induced Diabetes: Cytomegalovirus and Multiple Environmental Insults

Dear Sir,

Several viruses can cause diabetes in mice [1]. Encephalomyocarditis (EMC) virus infects pancreatic β cells and produces a severe form of diabetes in certain inbred strains of mice [2]. Under some circumstances, reovirus also infects β cells, and in addition, triggers an autoimmune response resulting in a mild form of diabetes characterized by abnormalities in glucose tolerance tests which generally disappear within several weeks [3, 4]. Diabetes may also result from cumulative β cell damage produced by sequential environmental insults. For example, in mice, a sub-diabetogenic dose of streptozotocin reduces the β cell reserve and enhances the capacity of certain viruses (e.g., Coxsackie B viruses) to produce diabetes [5, 6]. The present experiments show that murine cytomegalovirus (CMV) infection leads to mild focal β cell damage, and that multiple environmental insults can increase the severity of diabetes.

Newborn NIH Swiss male and female mice were infected with the Smith strain of CMV [7] or reovirus type 1 that had been passaged more than seven times in mouse pancreatic islet cell cultures to increase their tropism for β cells [4]. Two or four weeks later, the mice were challenged with a sub-diabetogenic dose of streptozotocin (1 mg/mouse) [5, 6], or the D variant of EMC virus [2], or CMV. Beginning one week after the challenge, each mouse was bled four times, and the glucose index and percentage of animals developing diabetes were determined as described previously [2].

Table 1 shows that when NIH Swiss male mice were infected with only CMV or reovirus, or given only streptozotocin, diabetes did not

Table 1. Glucose abnormalities produced by multiple environmental insults

First infection[a]		Challenge			Male			Female		
CMV	Reovirus	Strepto-zotocin	EMC virus	CMV	Number of mice	Glucose index[b]	Percent diabetic[c]	Number of mice	Glucose index	Percent diabetic
–	–	–	–	–	11	164 ± 17	0	10	141 ± 13	0
+	–	–	–	–	22	153 ± 16	0	20	124 ± 34	0
–	+	–	–	–	28	137 ± 19	0	25	133 ± 19	0
–	–	+	–	–	30	178 ± 10	0	32	152 ± 12	3
–	–	–	+	–	32	256 ± 81	56	10	182 ± 83	20
+	–	+	–	–	25	313 ± 88	84	27	253 ± 45	93
+	–	–	+	–	14	373 ± 70	100	22	214 ± 43	81
–	+	+	–	–	15	255 ± 34	93	10	204 ± 54	80
–	+	–	+	–	15	446 ± 112	100	23	303 ± 121	96
–	+	–	–	+	19	185 ± 29	5	23	164 ± 26	22

[a] NIH Swiss mice were infected with 2×10^5 PFU of CMV or reovirus at 2 to 4 days after birth. The animals were challenged 4 weeks later with 1 mg of streptozotocin or 1×10^4 PFU of EMC virus, or 2 weeks later with 2×10^5 PFU of CMV. [b] Each mouse was bled four times beginning one week after challenge and the glucose index (mean ± SD) determined. [c] Percentage of mice with a glucose index 3 SD above the mean of uninfected controls (i.e., males 164 ± 17%, females 141 ± 13%)

develop. In contrast, when males were infected first with CMV or reovirus and then challenged with streptozotocin, 84% and 93%, respectively, of the animals developed diabetes. Similarly, when males were infected first with CMV or reovirus and then challenged with EMC virus, the percentage of animals developing diabetes increased from 56% with EMC virus alone to 100%. In contrast, mice first infected with reovirus and then challenged with CMV virus showed a relatively small increase in the glucose index as compared with controls. The reverse experiment was not done because older mice are less susceptible to reovirus infection [8].

As seen in Table 1, the trend in female mice was similar to that in male mice, except that the glucose indices were generally lower [2]. The duration of the diabetes in the various groups (males and females) has not been determined. To be sure that the effects we were observing were due specifically to CMV or reovirus, and not accidental cross-contamination of the virus pools, mice were inoculated with the CMV or reovirus pools. Two weeks later, the animals were bled and sera were tested for neutralizing antibody to the homologous and heterologous viruses. These and other experiments failed to reveal any evidence of cross-contamination.

It is known that in mice CMV infects pancreatic acinar cells, but there is little information about the effect of the virus on islets [19]. In man, inclusion bodies are sometimes found in the islets of Langerhans of patients suffering from overwhelming CMV infection [10]. In the present study, histopathological examination of islets from CMV-infected mice revealed focal cellular infiltrates and pyknotic nuclei in a small percentage (i.e., 5%–15%) of the islets at 7–14 days after infection. Staining of the islets with a fluorescein-labelled anti-CMV antibody revealed viral antigens in 10% or less of the islet cells. The mild and focal islet cell damage produced by CMV does not appear to be sufficient to leave the mice diabetic at the end of 1 month, unless the animals are challenged with a virus or chemical that adds to the degree of β cell damage. Similarly, the previously reported glucose tolerance test abnormalities induced by reovirus generally disappear within 30 days [3, 4]. The fact that neither CMV or reovirus produces severe β cell damage may explain why only a few mice develop diabetes when first infected with reovirus and then challenged with CMV.

It should be emphasized that, in addition to the direct effect of viruses on β cells, certain viruses may trigger an autoimmune response against endocrine organs and/or result in a variety of hormonal changes which could contribute to glucose abnormalities. This could very well be a factor in mice infected with CMV or reovirus, and subsequently challenged with more severe betatropic agents (i.e., streptozotocin, EMC virus). Regardless of mechanism, the present study in mice points to the possibility that environmental insults, occurring early in life, may leave residual damage and set the stage for the subsequent development of insulin-dependent diabetes mellitus.

Yours sincerely,
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