HUMAN AMNION AS AN ADJUNCT IN WOUND HEALING

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Summary Biopsy specimens from the beds of leg ulcers of fifteen patients were obtained before and after the application for 5 days of cultured human amnion. After amnion application there was considerable granulation tissue in the ulcer bed and microscopical evidence of thinned connective tissues, vessel development, more compact resolution of vascular basement membranes, and many more factor VIII granules within endothelial cells. These findings suggest the presence of angiogenic factors in human amnion and this could explain the hitherto unexplained success of amniotic membranes in surgical practice.

Introduction Wound healing is affected adversely by poor general health and several local factors such as inadequate blood-supply and chronic infections. If direct closure of the wound is impracticable, a healthy granulating wound bed which will close either by marginal epithelialisation or by autografting is desirable. The plethora of creams, powders, solutions, and dressings used to promote wound healing indicates the incomplete state of knowledge on this subject. Human amnion has been sporadically used, since at least 1910, to promote the formation of granulation tissues, and lately as a biological dressing for open wounds, including burns and chronic ulceration of the legs. However, the vascular events which are initiated by this therapy have not been investigated. We present histopathological and immunohistological evidence for the development of profuse granulation tissue in the ulcer bed after amnion application to leg ulcers.

Patients and Methods Extra-embryonic membranes were collected and maintained in tissue culture as described elsewhere. The amnion epithelium and its basement membrane were separated by blunt dissection from the underlying amniotic mesenchyme and chorion immediately before application to leg ulcers. These tissues demonstrated good viability for up to 3 weeks in culture.

Fifteen patients with chronic leg ulcers (of up to 40 years duration) which were refractory to conservative and surgical methods of treatment, including autografting in eleven cases, were admitted and kept on strict bed rest throughout treatment. Their surgical management is reported in an accompanying paper.

Before and 5 days after amnion application two adjacent biopsy specimens were obtained aseptically from the ulcer bed. One was placed in 10% buffered formalin for haematoxylin and eosin (H and E) and reticulin stains. The other was snap frozen in liquid-nitrogen-cooled isopentane, and cryostat sections were prepared on microscope slides without chemical fixation and processed for immunohistology. Rabbit monoclonal antisera to human factor VIII was obtained from the Netherlands Red Cross Blood Transfusion Service and a fluorescein-isothiocyanate (FITC) conjugate of sheep anti-rabbit immunoglobulin (IgG) was obtained from Burroughs Wellcome, Kent. Fluorescence microscopy was performed with a Zeiss Universal microscope fitted with an FITC interference primary filter, dark ground condensor, and a 520 nm barrier filter.

Results Gross appearance.—In most patients a clean, red, delicate layer of granulation tissue had formed throughout the wound bed after 5 days of amnion application. Some had a less exuberant response, but all wound beds produced punctate bleeding after moderate to firm rubbing of the surface with a gauze swab. When the superficial granulation tissue was removed the underlying surface also bled freely.

Histopathology.—H and E stains of biopsy specimen from the ulcer bed before amnion application showed few small vessels (fig. 1A), but biopsy specimens taken after application of amnion showed many capillaries (fig. 1B). Reticulin stains of ulcer-bed tissues before amnion application contained dense connective tissue that tended to isolate groups of thick-walled vessels with disordered endothelium; many of the vessels appeared not to be patent (fig. 2A). After 5 days of amnion application the connective tissue fibres were more delicate and the vessels seemed to be more numerous, evenly dispersed, thin-walled, and patent, and the endothelium was often more clearly defined (fig. 2B). Biopsy specimens taken before amnion application showed prominent infiltrates of polymorphonuclear leucocytes and mononuclear cells, often with the morphological characteristics of plasma-cells.

Immunohistology.—Before amnion was applied biopsy specimens contained very little factor VIII in the vascular endothelial cells (fig. 3A), and cells reacting with this antisera were diffusely distributed, often giving a dull fluorescence in the vessel wall and surrounding connective tissues. However, all biopsy specimens taken after 5 days of amnion application contained brilliant immunofluorescent granules within the endothelium, and many more vessels were identified (fig. 3B).
Fig. 1—Representative fields in H and E stained sections.

Paucity of vessels in a generally densely collagenised stroma in pre-amnion biopsy specimens (A) as compared with numerous widely patent vessels in a much less dense stroma in post-amnion biopsy specimen (B).

**Autografting.**—These results are reported in an accompanying paper.12

To obtain a controlled assessment of the effectiveness of the use of human amnion as an adjunct in wound healing, four patients were managed by more traditional approaches to leg ulcer therapy before they had amnion application and autografting.12 Neither the gross nor the microscopical appearance of their lesions after their initial treatment displayed the favourable features of healthy granulation tissue seen after 5 days of amnion application.

**Discussion**

Mammalian embryos come to lie within a fluid-filled sac that arises from extra-embryonic tissues. The sac is composed of two principal layers.17 The chorion (cytotrophoblast) which forms the outer aspect of the sac is in contact with maternal cells without being rejected.18 The inner aspect, that tissue used in our present studies, consists of the predominantly single-celled layer of amnion epithelium and its underlying basement membrane. This part of the sac is bathed by amniotic fluid without actually being in contact with maternal tissue.19

In our hands, the most striking effect of amnion on the healing of chronic leg ulcers was the development of new vessels as observed grossly, histopathologically, and immunohistologically by antisera to factor VIII as a marker of endothelium.20 Vessel growth-promoting factors are said to be produced by tumour cells21 and by incompatible donor lymphocytes in graft-versus-host reactions.22 Since human extra-embryonic membranes share structural and functional properties with certain tumour cells,21,24 the effects we observed may be due to amnion angiogenic factors acting on the capillary endothelium. Could these factors be isolated for use as therapeutic agents in wound healing?

Other possible features that might promote the effectiveness of amnion as a biological dressing are its reported bacteriostatic properties,23 a feedback regulation of procollagen synthesis by collagen products in the explanted amnion,25 and an as yet undefined contributory function of the intermediary culture step we use. The usefulness of amnion as a biological dressing for burns11 and chronic leg ulcers12 suggests that it may have a broader application.

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Requests for reprints should be addressed to W. P. F.

REFERENCES


References continued overleaf.
REVERSIBLE MILD DIABETES IN CHILDREN AFTER TREATMENT WITH CHLORPROPAMIDE

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Summary

In 9 patients with juvenile-onset chemical diabetes treated with oral chlorpropamide, oral or intravenous assessments of carbohydrate tolerance were made regularly three weeks after withdrawal of therapy. 6 patients with sequential intravenous tests achieved statistically significant reversal of their carbohydrate intolerance and have remained normal for an average of 5.6 years (range 1-11 years). 2 patients who subsequently required insulin therapy were maintained in remission for 3.5 years and 5 years, respectively. There appears to be a group of young patients with chemical diabetes who achieve significant remission with sulphonylurea therapy.

Introduction

SULPHONYLUREAS "cure" experimental diabetes in animals. 1 Fajans and Conn 2 have shown improvement in the glucose-tolerance tests of young patients with mild diabetes after a course of tolbutamide. Various reports have confirmed this. 3-4 Stowers 5 observed that patients aged under 35 with chemical diabetes had a better carbohydrate-tolerance response to long-term chlorpropamide than did older patients. The former group included a number of children aged 3 to 16 years.

We now describe the behaviour of carbohydrate tolerance in a group of patients with juvenile-onset chemical diabetes treated with oral chlorpropamide.

Patients and Methods

9 patients (5 girls and 4 boys) with an average age at presentation of 12.0 years (range 3-16) were reviewed (table). Each patient had an oral or intravenous glucose-tolerance test under basal conditions. The result of the intravenous test was expressed numerically as an increment index.6 This is a measure of the rate of fall of the increment of the plasma glucose over the mean fasting level. An increment index >2.97 was normal and >2.46 abnormal. Results between these levels were regarded as probably abnormal. All patients had chemical diabetes at the time of diagnosis. 7

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at presentation (yr)</th>
<th>Family history</th>
<th>Mode of presentation</th>
<th>Weight at presentation (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>+ve, mother on tablets</td>
<td>Glycosuria found by mother</td>
<td>34.5</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>-ve</td>
<td>Recurrent furunculosis</td>
<td>56.5</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>+ve, paternal grandpa</td>
<td>Polyuria 1 yr</td>
<td>37.8</td>
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<tr>
<td>4</td>
<td>16</td>
<td>+ve, mother, father, 2 hexos on tablets</td>
<td>Screening of children of diabetic parents</td>
<td>61.1</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>-ve</td>
<td>2 hexos on tablets</td>
<td>54.9</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>-ve</td>
<td>Glycosuria on routine examination</td>
<td>55.6</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>+ve, mother on tablets</td>
<td>Glycosuria on routine examination</td>
<td>24.9</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>-ve</td>
<td>Screening of children of diabetic mothers</td>
<td>37.5</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>-ve</td>
<td>Primary symptoms within months</td>
<td>45</td>
</tr>
</tbody>
</table>

All 9 patients were given dietary advice and started on chlorpropamide. 50-100 mg daily. At the weight of presentation 6 patients was less than 1 standard deviation above the 50th percentile, of that of 2 less than 2 SD, and that of 1 more than 2 SD above the 50th percentile. 6 Glucose tolerance was tested yearly after discontinuation of the chlorpropamide for three weeks in order to obviate any direct pharmacological effect. The dosage of chlorpropamide was adjusted according to the result. The mean dosage during the period of follow-up was 128 mg daily (range 50-375 mg). The mean follow-up period was 7.2 years (range 3-16 years). 4 patients had first-degree relatives with diabetes; all the affected relatives were treated with tablets and 1 possibly had maturity-onset diabetes of the young (MODY).

Results

The behaviour of each patient's carbohydrate tolerance is shown in table. 7 patients achieved reversal to normal tolerance. Of these, 6 have remained normal for an average of 5.6 years (range 1-11 years), 3 of them having been off chlorpropamide for 8.0 years on average. In the other patient diabetes developed after a period of 7 years with increasing weight and intermittently raised fasting plasma glucose levels. He defaulted and has recently returned to the clinic, weighing 118 kg. Oral glucose tolerance is now normal 4 years after the finding of overt diabetes. Throughout he has been free of diabetic symptoms and he is the only patient in this group to have exceeded 2 SD above the 50th percentile.

13. Burgos H, Faulk WP. The maintenance of amnionic membranes in culture.
16. Faulk WP, Himmar W. Recent developments in immunofluorescence. Progr...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Initial tolerance</th>
<th>Initial fasting plasma glucose (mmol/l)</th>
<th>Tolerance at reversal or failure</th>
<th>Duration of treatment (yr)</th>
<th>Daily dose of chlorpropamide (mg)</th>
<th>Time to reversal or failure (yr)</th>
<th>Duration of normal tolerance (yr)</th>
<th>Most recent tolerance</th>
<th>Total follow-up (yr)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oral test: 60 min, 12.1 mmol/l; 120 min, 8.3 mmol/l</td>
<td>Oral test: 60 min, 12.1 mmol/l; 120 min, 8.3 mmol/l</td>
<td>4.5</td>
<td>4</td>
<td>50</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.89 I.I.</td>
<td>5.2</td>
<td>4.08 I.I.</td>
<td>8</td>
<td>100-375</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>16</td>
<td>Defaulter clinic for 4 yr</td>
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<tr>
<td>3</td>
<td>1.76 I.I.</td>
<td>5.5</td>
<td>3.96 I.I.</td>
<td>1</td>
<td>200</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>12</td>
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<td>4</td>
<td>2.57 I.I.</td>
<td>4.1</td>
<td>3.65 I.I.</td>
<td>2</td>
<td>100</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>11</td>
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<tr>
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<td>3.9</td>
<td>4.33 I.I.</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>Moved from area</td>
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<tr>
<td>6</td>
<td>2.48 I.I.</td>
<td>3.9</td>
<td>3.55 I.I.</td>
<td>1.5</td>
<td>100</td>
<td>1.5</td>
<td>1.5</td>
<td>0</td>
<td>3</td>
<td>Default clinic</td>
</tr>
<tr>
<td>7</td>
<td>2.37 I.I. (Mean 2.39 I.I.)</td>
<td>6.2</td>
<td>3.47 I.I.</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.61 I.I.</td>
<td>5.2</td>
<td>1.15 I.I. (FPG 6.5 mmol/l)</td>
<td>5</td>
<td>500</td>
<td>5</td>
<td>...</td>
<td>...</td>
<td>5</td>
<td>Symptom-free for period of follow-up</td>
</tr>
<tr>
<td>9</td>
<td>Oral test: 90 min, 13.9 mmol/l; 120 min, 12.2 mmol/l; 1.78 I.I.; 6 mo later (Mean 2.19 I.I.)</td>
<td>Oral test: 90 min, 13.9 mmol/l; 120 min, 12.2 mmol/l; 1.78 I.I.; 6 mo later (Mean 2.19 I.I.)</td>
<td>5.5</td>
<td>3.5</td>
<td>375</td>
<td>3.5</td>
<td>...</td>
<td>...</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>

LI.=increment index. FPG=fasting plasma glucose. mmol/l=mmol/l.

during the follow-up period. Within the group the average time to reversal was 2.1 years (range 1-5 years). The patients who achieved reversal remained on chlorpropamide for an average of 2.6 years (range 1-8 years).

The remaining 2 patients progressed to insulin therapy. Their remissions lasted for 3.5 and 5 years, respectively.

6 patients who achieved reversal had sequential intravenous tests. The difference between the initial tolerance and reversal tolerance was highly significant by Student's t test (t=7.30, p<0.001). Similar significance was found between the initial tolerance and the most recent tolerance (t=6.38, p<0.001).

If the total of 8 patients who had sequential intravenous tests are considered—i.e., including the 2 patients who showed no reversal to normal and progressed to insulin therapy—the difference between initial and most recent tolerance was at the border of significance (t=2.9, 0.05>p<0.10).

**Discussion**

In 7 of 9 patients (78%) abnormal glucose tolerance was reversed after treatment with chlorpropamide. 6 patients who had sequential intravenous tests achieved and have maintained highly significant reversal of carbohydrate intolerance.

Rosenbloom et al.,4 in a summary of published reports, noted that two-thirds of 33 children treated with sulphonylureas for periods of 0.2-5 years improved or maintained their glucose tolerance. Among 25 patients aged 9-17 years at presentation Fajans et al.12 found that in 72% the mean glucose tolerance showed some improvement. This group had been treated with diet or diet plus sulphonylurea. The number in each treatment group was not disclosed. The whole group was followed for 1.7-19.3 years. Our observations of the fate of carbohydrate tolerance in young patients with chemical diabetes over a similar period of time (3-16 years) are thus in broad agreement.
2 of our patients remained symptom-free for 3.5 years and 5 years, respectively, before needing insulin therapy. They had normal fasting blood-sugar levels and a negative family history of diabetes.

C-peptide studies on insulin-dependent patients suggest there is some recovery of endogenous insulin secretion during the "honeymoon period." Measurable C-peptide levels have been found in juvenile-onset diabetes beyond the "honeymoon period." Sulphonylureas reduce the need for exogenous insulin. Pollock et al., using an artificial pancreas, reported that treatment with a sulphonylurea resulted in a smaller requirement of insulin from the artificial pancreas and increased C-peptide concentration.

Sulphonylureas may have contributed to prolonging the remission in our 2 patients by potentiating beta-cell function.

The longitudinal assessment of carbohydrate tolerance has been limited by the reliability of the tolerance test. The oral glucose-tolerance test gives variable results in the same patient at varying time intervals. The criteria of abnormality are also variable. Jackson et al., in 200 oral tests on normal children, found that at each point there was skewing about the mean to higher values. This may lead to overdosage of chemical diabetes if conventional criteria are used. Jackson et al. observed that the intravenous test was less sensitive for initial screening than the oral test using 1.75 g of glucose/kg of ideal body weight, but he found that the intravenous test was more reproducible, thus making it more suitable for longitudinal studies. The intravenous test also avoids the variability related to gastrointestinal absorption.

The increment index was chosen for this study because, with normal fasting levels in patients with minor degrees of carbohydrate intolerance, the increments observed, rather than the absolute levels, give a better fit to an exponential line.

No large comparative studies of oral-versus-intravenous tests have been done in the age group under consideration. Jackson recommends caution in the interpretation of data from oral tests because the metabolic changes that accompany growth are complex. Sutherland et al. have argued cogently the value of the intravenous test in pregnancy, in which metabolism is similarly complex.

The mechanism of the improvement in carbohydrate diabetes after treatment with a sulphonylurea has been controversial. Loubatières et al. noted a "beta cytotoxic effect"—that is, neogenesis of beta cells. Hypertrophy of the islets of Langerhans has been observed in obese human beings. Regeneration of islets after sulphonylurea therapy has been reported.

The classical effect of sulphonylureas in the initial stages of therapy is the stimulation of release of insulin from beta cells. This beta cytotoxic effect would tend to correct the "deficiency in the insulin secretory mechanism of the beta cell" which Sherwin and Felig regard as "the predominant or primary lesion in most forms of diabetes." With long-term therapy there is little or no change in total plasma insulin. In reviewing the effect of long-term chlorpropamide on insulin levels, Turner and Holman have suggested that "the same insulin production at lower glucose levels may indicate improved beta cell efficiency." Fajans et al. have reported that with improved tolerance mean plasma-insulin responses had not deteriorated after an average of 7.1 years and that there was a statistically significant rise in plasma insulin at 30 min in oral tolerance tests in a group treated with diet and sulphonylurea.

The early phase of insulin secretion is deficient in many patients with chemical diabetes. Sulphonylureas stimulate the first phase of insulin release.

Various extra-pancreatic effects of sulphonylureas have been reviewed by Lebovitz and Feinglos and Krall and Chabot. Sulphonylureas induce an increase in the number of insulin-receptor sites both in animals and in man. Extra-pancreatic effects improve carbohydrate tolerance and Reaven et al. suggest that "resistance to the action of insulin is the primary lesion in patients currently classified as having borderline glucose tolerance or chemical diabetes.

It seems likely that insulin deficiency and insulin resistance are present to varying degrees in the diabetic syndrome. Sulphonylureas have a beneficial effect on both aspects. The variable end result of treatment of chemical diabetes may well depend on the individual response to agents capable of restoring glucose homeostasis in whole groups of patients. We have used chlorpropamide in an attempt to determine whether any improvement in carbohydrate tolerance may be due more to pancreatic or extra-pancreatic effects (Mutch, Stowers, Dingwall-Fordyce, Murchison, and Bottazzo, unpublished).

The majority of observations in the actions of sulphonylureas have been made with ongoing therapy. Our results, as well as those of Fajans and the contributors to the symposium in chemical diabetes mellitus in childhood, were obtained when the patient had been off treatment for 1-2 days (if on tolbutamide) or 1-3 weeks (if on chlorpropamide). These observations suggest that the potential to produce remission does not depend on the immediate pharmacological effect of the sulphonylurea but represents a modification of the basic metabolic abnormality.

Our understanding of the natural history of juvenile-onset diabetes has undergone considerable revision in the past two decades. Tattersall defined a "mild familial diabetes with dominant inheritance", and the concept of MODY has gained general acceptance. When the disease follows a general benign course the exact contribution of a particular therapeutic measure is much more difficult to establish. Rosenbloom et al. acknowledged the improvement of carbohydrate tolerance in several groups of children treated with a sulphonylurea but suggested that no investigator could truly distinguish between drug effects and natural history. Fajans was, however, careful to say that in his group he had established that there is not spontaneous fluctuation or improvement in carbohydrate tolerance prior to initiation of sulphonylurea therapy. Our results and the experience of others leads us to believe that there is a group of patients with chemical diabetes who may achieve remission from their carbohydrate intolerance for long periods after stopping sulphonylurea therapy. Fajans has expressed a similar opinion. Further information can only be gained by purposeful seeking for diabetes in its chemical state, which is the only one which sulphonylurea has been shown to benefit.

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Requests for reprints should be addressed to J. M. S.

REFERENCES

LARGE-BOWEL CANCER IN MARRIED COUPLES IN SWEDEN
A Follow-up Study

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Summary

The cause of death in 1951-1977 and cancer morbidity in 1959-73 were determined in 1094 (99.6%) eligible spouses of 1716 persons in Sweden who died from colon and rectum cancer in 1961. The risk of colorectal cancer and other possibly aetiologically related diseases was higher in the spouses than in a matched population.

Introduction

Epidemiological evidence and animal studies suggest that diet is important in the aetiology of large-bowel cancer. Bile acids and their degradation products, secreted in response to the consumption of fat, are believed to promote cancer whereas dietary fibre has been claimed to protect against bowel cancer. Most of the evidence comes from studies of international variation in bowel-cancer rates with diet and the increase in risk among migrants who move from low to high incidence areas.

If diet is an important factor in the development of large-bowel cancer, then spouses of patients with these tumours may be assumed to constitute a high-risk group since married couples are likely to have a similar diet for most of their adult lives. We therefore investigated the risk of colorectal cancer in spouses of patients who died from these diseases in Sweden in 1961.

Patients and Methods

1716 persons (index cases) were selected from the Swedish Central Bureau of Statistics as having died from cancer of the colon (ICD 153) or cancer of the rectum (ICD 154) in Sweden in 1961 (table I). Since 1947 a record of life-events from birth to death has been kept for every inhabitant of Sweden. Up till 1968 these records were stored in the archives of the Central Bureau of Statistics after a person's death.

Patients who had died before 1951 were excluded from the study, because reliable data on cause of death were not available before then. Persons who had divorced before 1947 were also excluded because they could not be indentified with certainty. The remaining 1098 spouses eligible for follow-up (table I), were sought in national population, death, and cancer registries. Cause and date of death were ascertainment between 1951 and 1973 and surviving spouses were identified as being alive on Jan 1, 1978. Cancer morbidity was ascertainment for the period 1959-1973; cancer registration was started in 1958 and had not yet been completed for the years after 1973.

Record linkage was based on the personal identification numbers introduced in Sweden in 1947. Only 4 of the 1098 persons could not be traced or had emigrated; 99.6% of the spouses eligible for study were identified.

International Classification of Diseases (ICD) codes assigned to patients were abstracted from the death register (ICD 6, 7 and 8) and from the cancer registry (ICD 7). Mortality was analysed according to the A-list for selected causes of death.
Person-years-at-risk were calculated by the life-table method for 1951-1977 for mortality and 1959-1973 for cancer morbidity. The numbers of deaths by cause and the numbers of cancer cases arising in this group of persons (expected numbers) were calculated on the basis of Swedish national mortality and cancer incidence rates, respectively, to derive mortality and morbidity ratios indirectly standardised for age, sex, and period of diagnosis.

The statistical significance of differences between the observed and expected number was determined. A Poisson distribution was assumed. 95% confidence limits of the mortality and morbidity ratios were estimated by dividing the true 95% confidence limits of the observed number by the expected number of cases.

### Results

**Colorectal cancer.** — The observed and expected number of deaths from colon and rectal cancer among spouses before and after 1961, the year of death of the index cases, are shown in table II. During the total follow-up period, 1951-1977, there were fewer deaths than expected (O/E = 13/18.33), but this difference is not statistically significant and may be due to random variation of small numbers. Whereas the risk in surviving spouses was the same as that in the general population, there was a deficit of cases before death of the index case.

In the A-list of the ICD, cancers of the small bowel are included with colon tumours. The error introduced by this is negligible in the estimate of expected numbers of colon cancer, no small-bowel tumours were observed.

The above findings are confirmed by the analysis of cancer registry data covering the years 1959-73 (table III). These data give more reliable information on the site of tumour than mortality statistics. None of the differences is statistically significant; this finding also applied when mortality and incidence data for both sexes were examined separately.

**Other causes of death**—Cancer of the large bowel may be aetologically related to cancer of the breast, ischaemic heart-disease, and gallbladder disease.10 We found no increased risk of death from these diseases among spousal of persons with large-bowel cancer (table IV), although the mortality ratio for ischaemic heart-disease 1.21 (1.02 – 1.42; p < 0.05) was higher in wives. The risk in men was not increased (SMR = 0.99). Standardised mortality ratios (SMR) for several other cancers and other causes of death had values around 1; none of the differences in mortality between spouses and total population was statistically significant (table IV).

### Discussion

We found no indication that the risk of colorectal cancer was increased among spouses of persons with large-bowel cancer (table II and III); nor was there an increased risk of diseases which have been suggested to be aetiologically related to cancer of the large bowel (table IV), except for the marginal increase in heart-diseases among women.

Although the comparisons were adjusted for sex, age, and period of diagnosis, no account could be taken of the fact that the present cohort consists of married persons only, because data on mortality by marital status are not available in Sweden. Data for England and Wales11 indicate that the mortality from colorectal cancer is

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**Table I—Deaths from colorectal cancer in Sweden in 1961 and reasons for exclusion from follow-up**

<table>
<thead>
<tr>
<th>Index person</th>
<th>Colon</th>
<th>Rectum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>No. of deaths 1961</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusions: Never married</td>
<td>82</td>
<td>109</td>
<td>46</td>
</tr>
<tr>
<td>Spouse died before 1951</td>
<td>56</td>
<td>126</td>
<td>38</td>
</tr>
<tr>
<td>Divorced</td>
<td>19</td>
<td>24</td>
<td>9</td>
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<tr>
<td>Follow-up: Eligible for follow-up</td>
<td>423</td>
<td>303</td>
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<td>Lost to follow-up</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Followed up 1951-1977 (1' of eligible)</td>
<td>423</td>
<td>300</td>
<td>262</td>
</tr>
</tbody>
</table>

---

**Table II—Deaths from colon and rectum cancer 1951-1977 in spouses of people (index cases) dying from these diseases in Sweden 1961**

<table>
<thead>
<tr>
<th>Cause of death in index case</th>
<th>Cause of death spouse</th>
<th>1951-60</th>
<th>1956-77</th>
<th>1957-77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Colon</td>
<td>2.72</td>
<td>5.55</td>
<td>8.27</td>
</tr>
<tr>
<td>Rectum</td>
<td>Rectum</td>
<td>1.50</td>
<td>2.72</td>
<td>4.22</td>
</tr>
<tr>
<td>Colon*</td>
<td>Colon*</td>
<td>1.37</td>
<td>2.56</td>
<td>3.93</td>
</tr>
<tr>
<td>Rectum</td>
<td>Rectum</td>
<td>0.71</td>
<td>1.20</td>
<td>1.91</td>
</tr>
<tr>
<td>Colon + rectum</td>
<td>Colon + rectum</td>
<td>6.30</td>
<td>12.03</td>
<td>18.33</td>
</tr>
</tbody>
</table>

* Includes cancer of the small intestine (ICD 152).
† Adjusted for age, sex, and period of death.
‡ Observed number significantly smaller than expected p < 0.05.

---

**Table III—Incidence of colon and rectal cancer in 1959-73 among spouses of persons dying from cancer of colon and rectum in Sweden in 1961**

<table>
<thead>
<tr>
<th>Cause of death in index case</th>
<th>Cancer mortality in spouse</th>
<th>Obs.</th>
<th>Exp.</th>
<th>O/E</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Colon</td>
<td>6</td>
<td>6.44</td>
<td>0.93</td>
<td>0.34-2.03</td>
</tr>
<tr>
<td>Rectum</td>
<td>Rectum</td>
<td>1</td>
<td>2.17</td>
<td>0.46</td>
<td>0.01-2.57</td>
</tr>
<tr>
<td>Colon</td>
<td>Colon + rectum</td>
<td>5</td>
<td>3.16</td>
<td>1.58</td>
<td>0.51-3.69</td>
</tr>
<tr>
<td>Rectum</td>
<td>Rectum</td>
<td>0</td>
<td>1.67</td>
<td>0.74</td>
<td>0.00-2.21</td>
</tr>
</tbody>
</table>

---

**Table IV—Causes of death 1951-1977 among spouses of people (index cases) dying from cancer of colon and rectum in Sweden 1961**

<table>
<thead>
<tr>
<th>ICD 8</th>
<th>Cause of death</th>
<th>Obs.</th>
<th>Exp.</th>
<th>O/E</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>Ca. esophagus</td>
<td>3</td>
<td>2.22</td>
<td>1.35</td>
<td>0.28-3.99</td>
</tr>
<tr>
<td>151</td>
<td>Ca. stomach</td>
<td>23</td>
<td>21.99</td>
<td>1.04</td>
<td>0.74-1.68</td>
</tr>
<tr>
<td>152,153</td>
<td>Ca. colon*</td>
<td>19</td>
<td>12.20</td>
<td>1.58</td>
<td>0.93-2.51</td>
</tr>
<tr>
<td>154</td>
<td>Ca. rectum</td>
<td>3</td>
<td>6.13</td>
<td>0.49</td>
<td>0.10-1.43</td>
</tr>
<tr>
<td>162</td>
<td>Ca. lung</td>
<td>6</td>
<td>8.61</td>
<td>0.70</td>
<td>0.26-1.52</td>
</tr>
<tr>
<td>174</td>
<td>Ca. breast (women)</td>
<td>8</td>
<td>1.74</td>
<td>4.69</td>
<td>0.87-10.21</td>
</tr>
<tr>
<td>185</td>
<td>Ca. prostate</td>
<td>12</td>
<td>10.79</td>
<td>1.13</td>
<td>1.07-1.94</td>
</tr>
<tr>
<td>410-414</td>
<td>HLD</td>
<td>256</td>
<td>231.84</td>
<td>1.10</td>
<td>0.97-1.29</td>
</tr>
<tr>
<td>574,575</td>
<td>Carcinoma colutatum, colorectum</td>
<td>4</td>
<td>7.04</td>
<td>0.57</td>
<td>0.15-1.45</td>
</tr>
<tr>
<td>Other causes</td>
<td>389</td>
<td>391.78</td>
<td>0.99</td>
<td>0.90-1.10</td>
<td></td>
</tr>
<tr>
<td>All causes</td>
<td>716</td>
<td>703.37</td>
<td>1.02</td>
<td>0.95-1.10</td>
<td></td>
</tr>
</tbody>
</table>

* Includes cancer of the small intestine (ICD 152).
† Adjusted for age, sex, period of death.
HLD = ischaemic heart-disease.
slightly lower in married women (standardised mortality ratio [SMR] 90-95%) than in the total female population. If this relation applies in Sweden and also to married men, then the expected numbers for colorectal cancer must have been slightly overestimated. However, multiplication of the expected numbers for colon and rectum cancer in tables II and III by factor of 0.9 i.e., the SMR for married women in England and Wales, would not change our conclusions. The close correspondence between the observed and expected numbers of deaths from causes not suspected to be related to colorectal cancer (table IV) strengthens our confidence in the validity of the method we used.

Several studies with this disease have shown an increased risk of colorectal cancer in first-degree relatives of patients. Macklin claimed that this risk of large-bowel cancer was not increased in spouses of patients with the disease. Wolfl et al. found that large-bowel polyps were more common in members of families at high risk of colon cancer (26 out of 55 persons) than in the spouses of patients with colon cancer. Only 1 questionable polyp was found in 25 of spouses. Chen et al. found no husband-wife pairs with colorectal cancer in a study in which 1.3 cases were expected. However, Lovett found that 3 out of 27 deaths among spouses were due to this disease. No firm conclusions could be drawn from these studies.

We have no proof that spouses in our study did indeed share the same diet throughout married life. However, it seems reasonable to assume a common dietary pattern in married couples.

The association of colon cancer with diet may be so weak and the variation in diet in Sweden so small that host factors or susceptibility may be the major determinants for the development of such cancer in Swedes given a certain level of environmental exposure. In a dietary survey with a one-week duplicate-portion technique, the range in average daily fat intake was 82.123 g in men and 47.92 g in women. An explanation of the present results based on the assumption that environmental exposure is uniform, does not exclude, for example, fat, which correlates with the international variation in bowel cancer, as a potential aetiological factor. Nor does it conflict with the changes in mortality from colon cancer observed in immigrants who may experience extreme changes in dietary habits and it also accords with the lack of association which emerged from several case-control studies in which attempts were made to relate colorectal cancer to diet i.e., the results may be due to limited variation in the diet of the population studied. Further investigations are needed to determine whether uniform exposure is the likely explanation for these "negative" findings. If such an explanation proves to be true, international studies will be a valuable means of testing hypotheses on diet and large-bowel cancer.

The possibility that diet in adult life is unrelated to the development of cancer of the colon should be considered. Three out of four other smaller studies of bowel cancer among spouses accord with this suggestion, but the finding of an increased colon cancer mortality in first-generation Japanese migrants to the U.S.A. does not. The results of our study do not exclude the possibility that premarital (childhood or adolescent) diet may be involved in the aetiology of cancer of the colon. This might also explain part of the increased risk among first-degree relatives, especially as there is no evidence that genetic factors can account for much of the variation in colon-cancer risk.

Our results require confirmation by others. If eating a diet identical with that of patients with bowel cancer is not associated with an increased risk, the current view of colon cancer aetiology may need to be revised and dietary patterns before marriage investigated. Studies of the risk in sibling relationships would be important approach.

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REFERENCES