Horsfall mucoprotein increases (Patel, 1964), as does the concentration of electrolytes, and the pH of the urine declines, so that here, too, conditions prevail under which casts will form.

Since there was no demonstrable increase in the urinary excretion of Tamm-Horsfall mucoprotein after the administration of ethacrynic acid, the formation of casts cannot have been due to the presence of raised mucoprotein concentrations in the urine.

The frequently observed spiral structure of the casts can be explained by the fact that Tamm-Horsfall mucoprotein has a molecular weight of 7×10^6 , and is about 5,600 Å in length and 42 Å in width (Tamm *et al.*, 1955). Molecules of this description are known to have a tendency to form thread-like, spiral aggregates.

The excretion of casts also persisted throughout the course of several days' treatment with ethacrynic acid and frusemide; it is therefore not due to an initial flushing effect. Similar findings, incidentally, were reported 20 years ago during long-term therapy with ion-exchange resins (Friedman *et al.*, 1951). The diuretics in question have all been in widespread use throughout the world for many years, and even during protracted treatment there has never been any indication that they might impair kidney function. It therefore seems to us that no

pathological significance can be attached to the presence of casts in the urine after the administration of diuretics. On the other hand, our findings would seem to indicate that the diagnostic value of hyaline casts is severely limited, unless special analytic methods (Rutecki *et al.*, 1971) can be used to determine their exact composition.

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PRELIMINARY COMMUNICATION

Insulin Infusion Test of Gastric Acid Secretion

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Summary

In view of the disadvantages of the standard insulin test for completeness of vagotomy a continuous insulin infusion test has been developed. Twenty-six tests were carried out on 12 healthy male volunteers. A dose of 0.04 unit of soluble insulin/kg/hr was found to produce the highest acid secretion with the least severity of symptoms of hypoglycaemia. The pattern of response was a plateau of acid secretion and of hypoglycaemia, and repeated studies in six subjects showed that the results were reproducible. Evaluation of this test after vagotomy and comparison with the standard insulin test are now required.

Introduction

The conventional insulin test to confirm completeness of gastric vagal denervation, although clinically valuable, remains potentially dangerous (Read and Doherty, 1970) and controversial

in its interpretation (Bank, Marks, and Louw, 1968; Gillespie, Elder, Gillespie, Kay, and Campbell, 1970).

Re-evaluation of the test led us to consider the use of a continuous insulin infusion technique aimed at producing a steady-state acid secretion in response to a steady level of hypoglycaemia.

We report here our initial experience with a continuous insulin infusion test in a limited number of healthy adult subjects.

Materials and Methods

A total of 26 tests were carried out on 12 healthy male volunteers with no previous history of dyspepsia and ranging in age from 22 to 35 years.

After a 12-hour overnight fast a nasogastric tube was passed into the stomach to allow ready recovery of a trial 20-ml instillate of water after discarding the fasting residue (Hassan and Hobsley, 1970). The tube was then connected to a vacuum pump for continuous gastric aspiration, which was interrupted every five minutes by air insufflation to ensure patency of the gastric tube. After two 15-minute samples of basal secretion had been collected the continuous insulin infusion was started. Soluble insulin (*B.P.*) was diluted in 154-mM NaCl to a total volume of 40 ml and delivered intravenously by a constant slow injection pump for three hours. Five different doses of insulin ranging from 0.025 to 0.10 U/kg body weight/hr were investigated.

Venous blood for glucose determination was obtained, one sample being withdrawn before starting the infusion and 10 samples at 15-minute intervals after its initiation. A glucose oxidase method and an autoanalyser were used for this purpose.

Gastric aspiration was continued for three hours with the collection of 12 15-minute samples. Each sample was measured for volume (ml), acid concentration (mEq/l.), and acid output (mEq). All titrations were made to pH 7.0 with 0.1 N NaOH in an automatic titrator (Radiometer, Copenhagen).

Throughout the test an E.C.G. tracing was taken every 15 minutes from standard lead II.

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A crude symptom score (see Table) was devised to assess the unpleasant symptoms experienced during the test. At the end of each 15-minute period the subject was given a score of 0 to 4 according to the severity of symptoms. Thus in a three-hour test a minimum total score of zero or a theoretical maximum score of 48 was possible.

Symptom Score

Score	0	1	2	3	4	Symptoms
0	..	..	..	..	..	None
1	..	..	..	..	..	Warmth, malaise, hunger*
2	..	..	..	..	..	Sweating, tremor (minimal)†
3	..	..	..	..	..	Sweating, tremor (severe)†
4	..	..	..	..	..	Coma†

* Subjective symptoms.

† Objective symptoms noted by person performing the test.

Results

TERMINOLOGY

The gastric secretory data are expressed as follows: (1) the basal acid output, being the sum of the two basal 15-minute collections multiplied by two and expressed as mEq/hr; (2) the peak acid output during insulin infusion, being the sum of the two highest consecutive 15-minute outputs during infusion multiplied by two and expressed as mEq/hr; and (3) the plateau acid output during infusion, being the sum of the four highest consecutive 15-minute outputs and expressed as mEq/hr.

With regard to blood glucose values the following terms are used: (1) the lowest blood glucose, being the lowest recorded level of blood glucose during the test; and (2) the plateau blood glucose, being the mean of all readings during the second and third hours of infusion. All of these values are expressed as mg of glucose/100 ml of blood.

PATTERN OF GASTRIC SECRETORY RESPONSE

The initial aim was to define the optimum dose of insulin that could be used in an infusion technique.

The response to insulin infusion in one subject is shown in the Chart. After an initial inhibitory period, which was not seen in all subjects, the volume, acid concentration, and acid output of the gastric aspirate increased. The acid concentration rapidly reached a plateau which persisted for the remainder of the test. The timing of the characteristic secretory response varied with the insulin dose, appearing as early as 45 minutes after beginning the infusion with the higher doses, and as late as 75 to 90 minutes with the lower doses.

The blood glucose level fell steadily throughout the first hour of infusion, the lowest reading being obtained in most subjects at the end of the first hour. Thereafter the blood glucose level remained almost constant.

Arrhythmias were never noted on serial E.C.G. recordings in any test.

INSULIN DOSAGE

0.10 U/kg/hr (two tests in one subject).—Both tests were associated with profound prolonged hypoglycaemia (plateau blood glucose 5 mg/100 ml) and severe unpleasant symptoms. The mean plateau acid output was 22.5 mEq/hr. This dose was not thought suitable for further evaluation.

0.05 U/kg/hr (two tests).—Again undesirable side effects were severe, with a plateau blood glucose of 10 mg/100 ml in one subject. The mean plateau acid output was 29.0 mEq/hr.

0.04 U/kg/hr (11 tests).—This dose occasioned fewer and less severe symptoms than the previous two doses. In seven tests the symptom score was less than six, and the mean symptom score of seven compares most favourably with that found during

administration of higher doses (26 with 0.1 U/kg/hr, and 18 with 0.05 U/kg/hr). In two tests hypoglycaemia was profound (plateau blood glucose 8 and 10 mg/100 ml respectively) but the fasting period had been prolonged, a fact reflected in the low fasting glucose levels of 40 and 45 mg/100 ml respectively. The mean symptom score in the remaining nine subjects was 4.5, the mean fasting glucose level was 65.5 mg/100 ml, and the mean plateau glucose level was 26.3 mg/100 ml. The mean plateau acid output in this group was 28.3 (S.D. 8.6).

Pattern of gastric secretory and hypoglycaemic response to insulin by continuous intravenous infusion.

0.03 U/kg/hr (10 tests).—Here the mean plateau blood glucose was 24.4 mg/100 ml and the mean symptom score 4. When the two subjects with prolonged fasting in the group tested with 0.04 U/kg/hr are excluded the two groups compare closely. At this dose the mean plateau acid output was 22.7 mEq/hr (S.D. 13.4).

0.025 U/kg/hr (one test).—At this dose the lowest blood glucose value was 43 mg/100 ml, with a plateau level of 44.5 mg/100 ml. This insufficient hypoglycaemic stimulus to gastric secretion is reflected in the relatively low plateau acid output of 13.3 mEq/hr in a subject who produced plateau outputs of 18.3 and 21.5 mEq/hr in two tests carried out with 0.04 U/kg/hr. Because of the uncertainty of the secretory stimulus this dose was not further investigated.

Two different doses were compared in four subjects, while in one four different doses were investigated. In every instance the acid output rose as the dose of insulin increased.

REPRODUCIBILITY

Six subjects underwent repeated insulin infusion tests with the same insulin dose. Four tests were repeated with 0.04 U/kg/hr and two with 0.03 U/kg/hr. When the acid output for a given

period in the first test was taken as 100% the mean ($\pm$ S.E. of the mean) change found in the second test was 13.25% ($\pm$ 6.0%) for the plateau acid output, 7.85% ($\pm$ 5.81%) for the peak acid output, and 13.88% ($\pm$ 9.14%) for the total acid output.

Discussion

Our data indicate that the administration of insulin by a continuous infusion technique does produce a steady-state acid secretion in response to a steady level of hypoglycaemia.

Although additional dose-response data will be required our initial trial indicates that a dose of 0.04 U of insulin/kg/hr produces the highest plateau of acid secretion associated with relatively few hypoglycaemic symptoms. It also appears important not to prolong the fasting period over 12 hours.

It is as yet too early to reach any definitive conclusions about the ultimate value of the continuous insulin infusion test, but it already appears safe and is associated with few side effects. It is hoped that by producing a steady state as opposed to a peak secretory response its interpretation will be facilitated.

The applicability of the criteria currently used for the conventional Hollander test (Hollander, 1946) and the definition of new criteria are at present being evaluated.

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MEDICAL MEMORANDA

Parathyroid Hormone Production and Malignancy

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We report relatively large amounts of parathyroid hormone found in extracts of a retroperitoneal sarcoma and in a hepatic metastasis from a carcinoma of the breast; neither tumour has previously been implicated in this syndrome (Omenn *et al.*, 1969). This hormone production by cancers of non-endocrine origin is now well recognized (Lipsett *et al.*, 1964).

Case Reports

Case 1.—A woman aged 52 presented with a lump in the outer quadrant of the right breast in February 1965. A radical mastectomy was performed and metastases in lymph nodes were found. After radiotherapy she was well until September, when pain developed in the right epigastrium and an enlarged liver was detected. Shortly after this she began to vomit and was admitted to hospital, where serum calcium was found to be 21.6 mg/100 ml, serum phosphorus 3.3 mg/100 ml, and serum alkaline phosphatase 26 K.A. units/100 ml. After treatment with intravenous fluids and corticosteroids the serum calcium fell slowly to a minimum of 12.0 mg then rose again to 14.5 mg/100 ml. No bony metastases were found radiologically. Her condition

deteriorated and she died on 1 November. Before her death serum phosphorus reached 5.5 mg/100 ml, serum alkaline phosphatase 52 K.A. units/100 ml, and blood urea 110 mg/100 ml. At necropsy, 28 hours after her death, the liver weighed 4,070 g and was heavily infiltrated with tumour. No metastases were found in any other organ, and none were found in bone or bone marrow. The two parathyroid glands found were normal. Histological examination of the liver showed many oval irregular epithelial cells forming irregular tubules and acini consistent with metastatic carcinoma from a primary breast tumour.

Case 2.—A man aged 61 was admitted to another hospital in December 1966 with dementia, slurred speech, and vomiting. Hypercalcaemia (13.5 mg/100 ml) was discovered and parathyroid exploration undertaken. Two normal parathyroid glands were found. In January 1967 a firm mass was found in the right loin and operation showed what appeared to be an inoperable carcinoma of the kidney. Hypercalcaemia persisted despite high doses of corticosteroids. He was admitted to the Royal Melbourne Hospital on 7 February, where he was found to be emaciated and confused. A large irregular mass was present in the right loin. Serum calcium was 21.4 mg/100 ml on admission and serum phosphorus ranged from 1.7 to 2.4 mg/100 ml. Treatment with intravenous sodium phosphate temporarily restored serum calcium to normal but it quickly rose again. Immediately before death serum calcium was 18.0 mg, serum phosphorus 4.3 mg, and blood urea 232 mg/100 ml, and serum alkaline phosphatase was 19 K.A. units. No lesions were detected radiologically in his bones. Necropsy two hours after death disclosed a huge haemorrhagic and necrotic retroperitoneal tumour invading the right kidney, adrenal, liver, and surrounding muscle. The vertebrae were not involved. Small nodular metastases were present in the lungs. Microscopically the tumour consisted of bands of long cells, often spindle-shaped and poorly defined.

Tissue Assay and Results

Normal or malignant tissue was obtained at necropsy or at operation and frozen. The frozen material was lyophilized, powdered, defatted with acetone, and dried under vacuum. Extraction was performed with phenol (Aurbach, 1959) or urea-hydrochloric acid-cysteine (Rasmussen *et al.*, 1964) to the stage of solvent precipitation, for which ether was used. The precipitate was washed with acetone, extracted with acetic acid, and immunoassayed. Antisera to parathyroid hormone were

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