The double glucagon test, a new liver function test

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Summary

A double glucagon test was performed in 100 patients and seventeen control subjects. Two intravenous doses of glucagon (1 mg) were administered at a 1-hr interval. Glucose was determined before the first injection and 20, 25 and 30 min after both doses, and the increases after each successive dose were added up.

In normal persons, the total increase in glycaemia was higher in the second than in the first hour and reached at least 110 mg. Normal values were found in cases of obstructive jaundice, cholelithiasis and adult diabetes, and abnormal values in patients with infective hepatitis, liver congestion and cirrhosis.

The double glucagon test was found to be very useful for the differential diagnosis of liver cell impairment and hepatic obstruction.

Introduction

Glucagon increases hepatic glycogenolysis by activating the phosphorylating enzymes by which glycogen is converted into glucose (Sutherland & Cori, 1951; Van Italy & Bentley, 1955). Considering

that the glycogen content of the diseased liver is usually low and that glucagon apparently does not induce muscular glycogenolysis but only hepatic glycogenolysis, the possibility of a new liver function test was envisaged.

Subjects

The study included 100 patients (fifty-three men and forty-seven women, age range 20-74 years), most of them with jaundice. Their diagnoses are presented in Table 1 and some additional information is given below. In three of the patients with obstructive jaundice the symptoms lasted for several weeks. Two of the patients with acute hepatitis displayed a cholestatic component with marked increase in alkaline phosphatase. Four of the nine patients with cholelithiasis had cholecystitis, but none of them suffered from obstructive jaundice. Patients with hepatic precoma were excluded because of the risk of severe hypoglycaemia which might speed the onset of coma.

In addition, a control group of seventeen persons was selected. All of them were free from hepatic

TABLE 1. Results of the double glucagon test in various diagnostic categories and in a control population. Minimum, maximum and mean increases in mg/ml in the first (Σ_1) and second (Σ_2) test as compared with the pre-glucagon control levels and the number of instances in which Σ_2 was lower or higher than Σ_1

Diagnoses	Number of patients	Σ_1			Σ_2			$\Sigma_1 > \Sigma_2$	$\Sigma_1 < \Sigma_2$
		Min	Max	Mean	Min	Max	Mean		
Obstructive jaundice	24	95	185	130	120	205	250	1	23
Acute infective hepatitis	21	0	110	73	10	75	51	19	2
Hepatocirrhosis	20	0	123	70	-10	175	64	15	5
Liver congestion due to heart failure	12	43	180	99	35	110	75	12	0
Cholelithiasis	9	98	182	134	112	205	148	1	8
Liver metastases	8	45	112	83	40	120	77	6	2
Adult diabetes	6	92	160	125	104	175	146	0	6
Control group	17	96	200	136	110	240	160	0	16*

* One case: $\Sigma_1 = \Sigma_2$.

disease and had normal serum flocculation tests, bromsulphthalein and serum transaminases.

Method

The tests were performed in the morning after a 14-hr fasting period. Two intravenous doses of 1 mg of glucagon were given at an interval of 1 hr. Capillary blood samples were taken, by finger prick, 5 min and immediately before the first injection, and 20, 25 and 30 min after the first and second injection. The capillary blood glucose (mg/ml) at the six post-glucagon determinations was compared, for each subject, with the control level (average of the two pre-glucagon samples). The sum of the increases after each injection indicated the 'total hyperglycaemia' in the first (Σ_1) and second (Σ_2) hour. Blood glucose was determined by the Nelson-Somogyi method (Nelson, 1944).

Results

The results for the various diagnostic categories and the control group are presented in the accompanying table.

In the control group, the mean average increase in glycaemia was 136 mg (range 96–200) in the first hour and 160 mg (110–240) in the second hour. In

sixteen subjects Σ_2 was higher than Σ_1 and in one subject Σ_2 was equal to Σ_1 .

A normal response to glucagon was observed in the patients with obstructive jaundice even when the disease had been present for several weeks.

In patients suffering from infective hepatitis, Σ_1 was lower than in the control group and the overlap was very slight. Σ_2 was always lower than in the control population and usually lower than Σ_1 (Fig 1A).

Similar results were achieved in patients with cirrhosis; the overlap was somewhat larger than in the patients suffering from hepatitis and two patients displayed a normal double-glucagon test (Fig. 1B).

The difference was statistically highly significant ($P < 0.0001$) for Σ_1 , Σ_2 and $\Sigma_2 - \Sigma_1$ between the control group and the patients with hepatitis, and for Σ_1 and Σ_2 between the controls and the patients with cirrhosis.

Patients with hepatic congestion due to heart failure showed low Σ_1 , and Σ_2 was lower than Σ_1 in all instances. Patients with liver metastases had similar low values in the two tests and Σ_2 was lower than Σ_1 in six of eight cases. As several patients were rather cachectic at the time of the test, malnutrition may have interfered with the results.

FIG. 1. Individual results of the double glucagon test. Summed increase in glycaemia after the first (Σ_1) and second (Σ_2) injection of 1 mg glucagon.

Neither cholelithiasis nor stable diabetes had any influence on either Σ_1 or Σ_2 , the results of the tests being very similar to those in the control group.

Discussion and conclusion

In the control group, the response to intravenous glucagon is higher after the second than after the first dose. This is probably due to the double action of glucagon on the production of glucose: a glycogenolytic action of rapid onset and a gluconeogenic effect starting after about one hour (Exton *et al.*, 1966). Thus the increase in blood glucose, in the second hour, is the sum of the glycogenolytic effect of the second injection and the gluconeogenic effect of the first. This hypothesis will be further investigated.

Recently we performed a double glucagon test before and after phenformin, since phenformin was reported to inhibit gluconeogenesis (Altschuld & Kruger, 1968). As expected, the total hyperglycaemia in the second hour (Σ_2) was lower after phenformin than before phenformin (Verhaegen *et al.*, 1970).

Ghannem *et al.* (1962) and Linke (1959) examined liver function by the single glucagon test and found a significant difference between patients suffering from liver diseases and normal persons. However, the great overlapping invalidated the value of the test. As the double glucagon test offers a larger spreading of the values with a different course according to various diagnostic categories it seems to possess a higher absolute and differential diagnostic value.

The test does not appear to be sufficiently informative for assessing the severity of cirrhosis as patients with well compensated cirrhosis generally presented abnormal values and the one patient with advanced alcoholic cirrhosis had a normal response.

Figure 1C shows that the results are widely divergent in cases of obstructive jaundice and hepatitis.

In the patients with obstructive jaundice and in those with cholelithiasis the pattern was very similar to that of the control population. Furthermore, the three patients with long-standing obstructive jaundice displayed clearly pathological serum flocculation tests and transaminases although the double glucagon test was unaffected.

All the patients with infective hepatitis displayed considerably divergent results from those of the control group. In many of these patients, the double glucagon test was repeated after a few weeks. The pattern remained abnormal until 6 weeks after the onset of the disease, although by this time all other biochemical parameters had returned to normal.

The follow-up studies are of too short a duration to establish whether the double glucagon test is effective for differentiating between patients in whom hepatitis develops into chronic hepatitis and those in whom the disease develops into cirrhosis.

Our results offer strong evidence for the value of the double glucagon test in the differential diagnosis of liver cell impairment and obstruction.

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