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The 2-Deoxy-D-Glucose - Neutral Red Test and Vagotomy

An Experimental Study

L. C. J. VAN RENSBURG

SUMMARY

The 2-deoxy-D-glucose - neutral red test proved to be successful in assessing completeness of vagotomy in the baboon both intra- and postoperatively. There were no deleterious side-effects and we found that both products could be sterilized adequately. So far we have used this test on 5 patients in the immediate postoperative phase; in 1 patient, on whom the surgeon thought he had done an incomplete parietal cell vagotomy, the test was found to be positive within a week of the operation.

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2-Deoxy-D-glucose (2-DG) is a powerful stimulus of vagal gastric secretion and has been employed both experimentally and clinically for some time.¹⁻⁴ Neutral red (NR), a basic dye, will appear in the lumen of the stomach after intravenous injection. The use of a combination of 2-DG and NR has proved to be a reliable test for the completeness of vagotomy.^{4,5} However, it has not been generally accepted, for there are suggestions that 2-DG is a liver toxin, that it could cause cardiac irregularity and that it cannot be adequately sterilized. The aim of the present study was to test its effectiveness and possible side-effects in the baboon. It might be added that very little work has been published on its use in association with highly selective vagotomy.

MATERIALS AND METHODS

Chacma baboons (*Papio ursinus ursinus*) with an average weight of 15 kg were used in this study. A sample of

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blood was taken on the day before the operation for liver function tests, serum electrolytes and serum glucose levels. All the operative procedures were done under anaesthesia with ketamine (Ketalar) 2 mg/kg body weight, 1% halothane (Fluothane) and oxygen. The baboons had to be anaesthetized for all postoperative intubations but not for the taking of blood samples. The following procedures were performed: group I — gastrotomy alone; group II — truncal vagotomy and pyloroplasty; group III — truncal vagotomy and gastro-enterostomy; group IV — truncal vagotomy and antrectomy; group V — highly selective vagotomy; and group VI — deliberate incomplete vagotomy.

Five baboons were allocated to each group except for group V, where the allocation was 10. During the operative procedure the ECG was monitored, the blood glucose and alkaline phosphatase levels were estimated every 15 minutes, and both these tests were repeated the following day. Liver biopsies were done in each group. The usual dose of 2-DG was 15 mg/kg body weight and all the animals received 15 mg NR. Each product was dissolved in 10 ml normal saline and passed through a membrane filter (Millipore). Subsequent cultures were always found to be sterile.

The 2-DG was administered intravenously by slow injection followed by NR 10 minutes later. It is important not to load the circulation with glucose during the operation, as this causes a delayed response to 2-DG. It is also important not to give the animals atropine as a premedication. Within 15 - 30 minutes after the injection of the stimulant and dye, a purplish red dye was present in the lumen of the stomach in the unvagotomized animals (group I). The presence of dye was seen on gastrotomy and in the aspirate from a nasogastric tube. Whenever the vagotomy was complete (groups II - V), no dye was found in the lumen of the stomach. When a deliberate incomplete vagotomy (group VI) was performed, the dye always appeared in the lumen of the stomach and it was also possible at gastrotomy to see which nerve, i.e. the anterior or posterior vagus, was still intact because there was a regional secretion of the dye. The usual procedure was as follows: once the vagotomy was complete the stimulant and dye were injected and the stomach was opened in the appropriate region for the various procedures, i.e. pyloroplasty, gastro-enterostomy and antrectomy. A large swab was then placed in the region of the parietal cells and no difficulty was experienced in detecting the presence of dye on the swab. If no dye was present it was assumed that vagotomy was complete. When a parietal cell vagotomy was performed the stomach was not opened, a soft bowel clamp was applied just proximal to the antrum, the stomach was irrigated with saline via a nasogastric tube and if no dye appeared in the aspirate within 20 minutes of the injection of 2-DG it was assumed that the vagotomy was complete. One week after the operative procedures the animals were intubated and the test was repeated. In the incompletely vagotomized animal the dye appeared in the aspirate within 15 - 20 minutes after the injection of the 2-DG. A month later the tests were repeated and similar results were found.

We found no change in the serum electrolytes, the ECG or liver biopsies. The blood glucose rose slightly after the injection of 2-DG. We did however find some variation in the alkaline phosphatase levels. Unfortunately the baboon has an unstable alkaline phosphatase level, making intra- and postoperative changes difficult to interpret. For this reason a control study was performed. The operating room temperature was on the average 24°C and the primates' temperature 37,5°C. The alkaline phosphatase levels were studied in the following groups: group Ia — ketamine 2 mg/kg body weight was given intramuscularly (Fig. 1); group Ib — the same procedure was performed but the baboon also received the usual doses of 2-DG and NR (Fig. 2); group IIa — ketamine 2 mg/kg body weight was given intramuscularly in addition to 1% halothane and oxygen (Fig. 3); group IIb — the same procedure was repeated but the usual doses of 2-DG and NR were also given (Fig. 4); group IIIa — ketamine, halothane and oxygen were

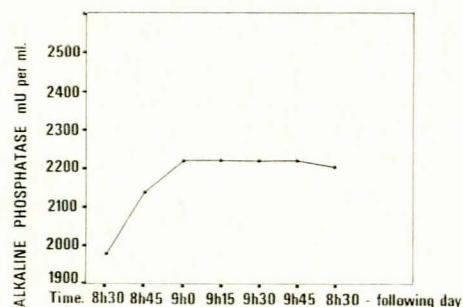


Fig. 1

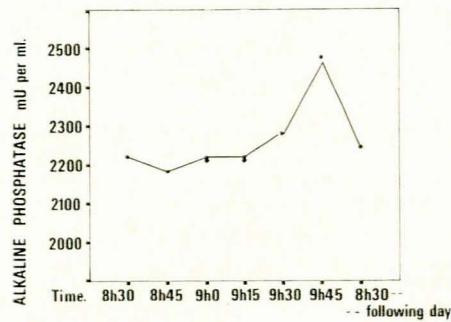


Fig. 2

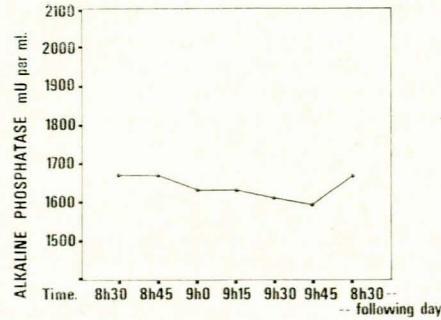


Fig. 3

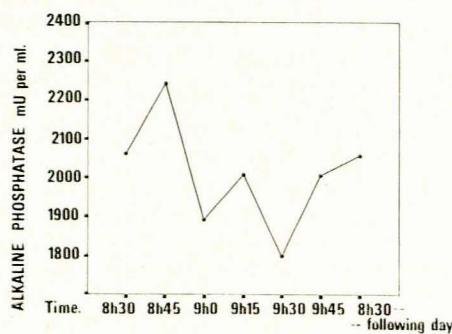


Fig. 4

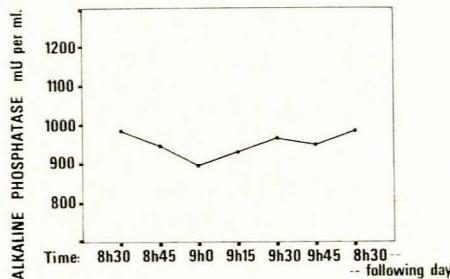


Fig. 5

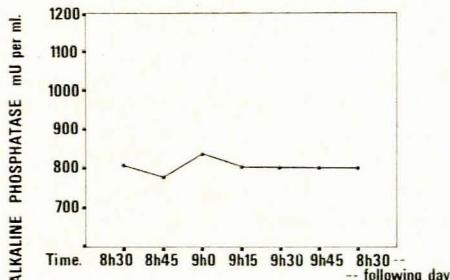


Fig. 6

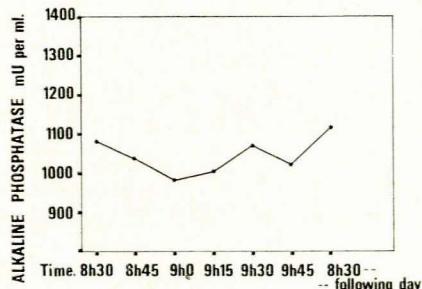


Fig. 7

given in the usual doses and a laparotomy and gastrotomy were performed (Fig. 5); group IIIB — the same procedure was performed but in addition the animal received 2-DG and NR (Fig. 6); group IVa — ketamine, halothane and oxygen were administered in the usual doses, but in addition a truncal vagotomy and pyloroplasty were performed (Fig. 7); group IVb — the same procedure

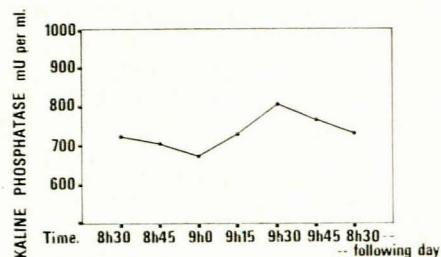


Fig. 8

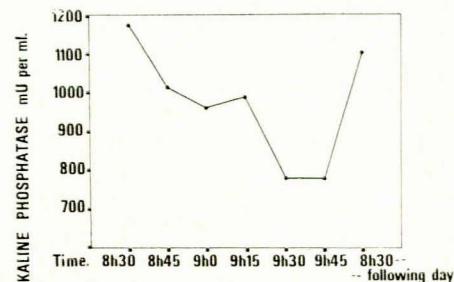


Fig. 9

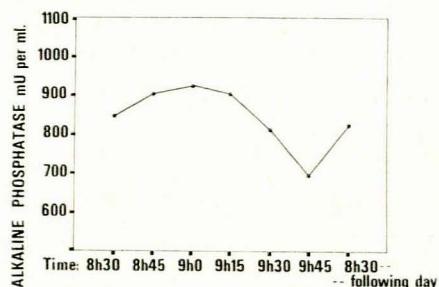


Fig. 10

was performed but in addition the animal received the usual doses of 2-DG and NR (Fig. 8); group Va — ketamine, halothane and oxygen were administered in the usual way and highly selective vagotomy was performed (Fig. 9); group Vb — the procedure was repeated, with the addition of the usual doses of 2-DG and NR (Fig. 10).

The average duration of anaesthesia was 1 hour. During the procedure the alkaline phosphatase in 5 specimens was estimated every 15 minutes and again 24 hours later. The accompanying charts are samples of a typical response and it will be noted that the addition of 2-DG and NR caused no significant change.

DISCUSSION

2-Deoxy-D-Glucose

In 1952 Cramer and Woodward⁸ reported that 2-DG prevented the growth of yeast cells because of an anaerobic glycolysis. Laszlo *et al.*⁷ studied the effects of 2-DG and 2-D-galactose on experimental tumours and found that

there was an inhibition of tumour growth in each instance; they suggested that the results were consistent with the hypothesis that there was interference with glycosis in the hexokinase reaction which could be inhibitory to tumour growth. Landau *et al.*⁸ studied the metabolic and pharmacological effects of 2-DG infusions on cancer patients. It was found that the blood sugar level rose after intravenous administration of 2-DG, and side-effects such as flushing, sweating, or drowsiness were transient and not severe. These symptoms were suggestive of a hypothalamic response which could be due to the inadequate glucose utilization by the brain cells causing symptoms as seen in hypoglycaemic reactions. Collin-Jones and Himsorth⁹ postulated that hypothalamic hypoglycaemia occurred which, in turn, stimulated the dorsal nucleus of the vagus. This response is cancelled by vagotomy, atropine and strong traction on the vagus nerves. Hirschowitz and Sachs,¹ and Duke *et al.*,² compared the vagus-stimulating effect of 2-DG with that of insulin and found 2-DG a more powerful stimulus to vagal gastric secretion and superior because of its reliability and reproducibility of the response. Thomas and Duthie³ came to the same conclusion. Some of their patients however, had side-effects such as an exaggerated hypoglycaemic response and hypothermia, and in 2 out of 37 there was evidence of mild liver damage. Because of the side-effects it has been suggested that this test should not be employed in patients over the age of 60, patients with known heart disease, patients on digitalis and patients with cerebrovascular abnormalities. Our experimental animals, however, showed no ECG abnormalities, evidence of liver damage or, as far as could be judged, cerebral changes.

Neutral Red

For many years various dyes have been used by physiologists and research workers in the study of gastric secretion in animals and also in man. Greenwood¹⁰ in 1884, in a study of the gastric glands of a pig, reported that the acid-secreting cells of the stomach could be stained with silver nitrate. Delrue¹¹ reported his findings on acid secretion studies in the isolated frog stomach and demonstrated that histamine increased the acid output. Henning¹² showed that frog gastric mucosa could secrete certain dyes *in vivo* and demonstrated secretion of NR from the 'fundic' gland. Glaessner and Wittgenstein¹³ were able to demonstrate that NR administered intravenously appeared in the lumen of the stomach in mice and also in humans. Bradford and Davies¹⁴ found that the isolated frog and toad gastric mucosa could transport a variety of dyes (including NR) from a nutrient medium into the lumen of the stomach. It has repeatedly been shown that NR is excreted only by the parietal cell area and not from the antrum.

The combination of these two products, one a stimulator and one an indicator, makes this an attractive and practical test of the completeness of vagotomy. In agreement with other workers, we found this test reliable in our experimental animals and also on a limited number of patients.

Very little has appeared in the literature on its use in association with parietal cell vagotomy. Eisenberg *et al.*¹⁵ made some interesting observations comparing the effects of 2-DG and insulin on acid secretion in dogs with gastric fistulae and Heidenhain pouches. They used 2-DG in doses of 50 - 200 mg/kg body weight and found this a strong stimulant of gastric acid secretion; it was of interest that acid was secreted from the Heidenhain pouches. They suggested that the denervated pouch was probably stimulated by the secretion of gastrin from the stomach and showed that this effect could be cancelled by instilling an acid solution into the antrum of the remaining stomach. In theory one is tempted to compare the parietal cell vagotomy model with that of a Heidenhain pouch model, since both procedures cause denervation of the parietal cell. Although Eisenberg did not use NR as an indicator, his findings in relation to the strong stimulatory effect of 2-DG are rather disturbing. Although it has been suggested that the vagal release of gastrin is of minor importance, as far as recurrent ulceration is concerned after a highly selective vagotomy,¹⁶ we felt that it would be important to demonstrate whether or not the injection of 2-DG could stimulate the nerves of Latarjet to such an extent with gastrin release that it could cause the parietal cells to secrete the NR in spite of a complete denervation of that area. In none of our cases where we felt that parietal cell vagotomy was complete was there any secretion of dye, even with a dose of 200 mg/kg body weight of 2-DG. However, we always found a positive response in the cases where we deliberately performed incomplete parietal cell vagotomy. We also wondered whether excessive traction on the vagi during a parietal cell vagotomy could cancel the effect of 2-DG. All our animals were re-examined 1 week later and the final result was similar to that found intra-operatively, i.e. there were no false negatives. We believe this test to be a reliable intra-operative one, the only drawback being that the operation is prolonged by 15 - 20 minutes.

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