L-asparaginase or whether their effects are quite independent. For example, it might be argued that those patients in whom the number of leukaemic cells in the bone marrow fell on treatment with Ara-C would have achieved remission with this drug alone had further doses been given. It was felt that to continue giving Ara-C to patients who were already grossly neutropenic would have involved a considerable risk of infection, while to withhold all treatment was unnecessary and unjustifiable when L-asparaginase was available. Indeed, our results illustrate that a major advantage of L-asparaginase over other antileukaemic drugs is that it may be used to treat patients in whom the bone marrow is severely hypoplastic without producing further marrow depression. They confirm the findings of other workers who have found that L-asparaginase is non-toxic to normal bone-marrow elements.

At a purely speculative level it may be suggested that the bone-marrow hypoplasia produced by Ara-C serves to enhance the effectiveness of L-asparaginase, since the removal of normal cells from the bone marrow depletes a source of the extrinsic L-asparagine which the leukaemic cells require. Since L-asparaginase is unable to penetrate cell membranes or enter the extracellular fluid it can only hydrolyse circulating L-asparagine, leaving the leukaemic cells with a second source of the amino-acid—that obtained by diffusion from those normal cells with which they are in direct contact in the bone marrow. Thus if the number of normal cells is reduced, further "starvation" of the leukaemic cells may result.

Another way in which Ara-C might increase the sensitivity of the leukaemic cells to the action of L-asparaginase is by synchronization of the mitotic cycle following inhibition of reproduction. This is known to occur with HeLa cells (Kim and Eidinoff, 1965), and a similar effect has been observed with leukaemic cells (Lampkin *et al.*, 1969), so it may be that synchrony permits greater cell kill by another antitumour agent to which the dividing cells are sensitive.

These preliminary results are encouraging enough to suggest the need for a controlled trial of such treatment, and this is now being undertaken. It would seem appropriate also to compare the effect of giving both drugs together, with their sequential use. If both these types of treatment were found to give equally satisfactory results, there would be a theoretical advantage in using the two drugs sequentially rather than together, since patients achieving remission on Ara-C alone would then be able to receive L-asparaginase for the first time in a subsequent relapse, either alone or after further Ara-C. This is illustrated by Case 4, who had received a course of Ara-C in a previous relapse and who had achieved complete remission on that occasion.

We should like to thank the physicians of the Hospital for Sick Children who have allowed us to study patients under their care and to participate in their management. We are grateful to Dr. H. B. Allen, of F.B.A. Pharmaceuticals Ltd., and Dr. C. E. Gordon Smith, the Microbiological Research Establishment, Porton Down, Salisbury, for supplies of L-asparaginase, and to Dr. W. P. Goodyear, of Upjohn Ltd., for cytosine arabinoside (cytarabine). The in-vitro asparaginase sensitivity tests were kindly carried out by Dr. D. Crowther and Mr. R. L. Sewell at the Chester Beatty Research Institute, Sutton. This work was supported by a grant from the Leukaemia Research Fund.

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Preliminary Communications

Proteins and Insulin Release: A Dual Role of Amino-acids and Intestinal Hormones

British Medical Journal, 1969, **4**, 598–600

Summary: In two subjects concurrent infusion of amino-acids and the hormones secretin and pancreozymin provoked much higher plasma insulin levels than did administration of amino-acids or hormones individually. It is suggested that this may be a physiological phenomenon, augmenting the release of insulin from the pancreas after a meal containing protein.

INTRODUCTION

The first, indirect, evidence that amino-acids might stimulate insulin release was provided by Mellinkoff *et al.* (1955). Subsequently, several authors have shown that, in man, amino-acids, whether given orally or intravenously, or protein meals increase the level of circulating plasma insulin (Berger and Vongaraya, 1966; Floyd *et al.*, 1966; Rabinowitz *et al.*, 1966). There is

considerable variation in the insulin response to individual amino-acids, arginine generally being the most potent stimulant. Certain amino-acids also stimulate insulin release in vitro (Malaisse and Malaisse-Lagae, 1969; Turner, 1969), so that one possible explanation of the insulinotropic action of protein foods is through direct pancreatic stimulation by the amino-acids resulting from digestion. Alternatively, the hormones secretin and pancreozymin, both secreted in response to protein foods and both known to stimulate insulin release, may be responsible, either alone or by interaction with amino-acids, for the insulinotropic effect. The latter possibility is the subject of the present investigation.

MATERIAL AND METHODS

Two volunteer subjects were investigated on several occasions after an overnight fast. While lying at rest constant infusions of (1) physiological saline, (2) amino-acids, (3) pancreozymin, (4) secretin, or (5) combinations of 1–4 were made into a vein of one arm; blood samples were collected from an indwelling venous catheter in the other.

Amino-acids were administered as a casein hydrolysate (Aminosol 10%), kindly provided by Dr. P. C. Allen, of Messrs. Paines and Byrne Ltd. Purified preparations of pancreozymin

and secretin were provided by Drs. Jorpes and Mutt, of the Karolinska Institute, Stockholm—pancreozymin/cholecystokinin (75 Ivy dog units of cholecystokinin and 300 Crick, Harper, and Raper units of pancreozymin per 0.03 mg. of active polypeptide; batch No. 26762) and secretin (75 clinical units/ampoule; batch No. 16781).

Amino-acids were infused at a rate of about 1 g. in 2.3 minutes, so that the total infused in 46 minutes was about 20 g. This corresponds to the quantity of amino-acids derived from a moderately sized steak. Secretin was infused at a rate of 7.5 clinical units/2.3 minutes and pancreozymin at 1 μ g. active polypeptide/2.3 minutes.

Blood was taken into tubes containing edetic acid, and the plasma separated by centrifugation. Plasma immunoreactive insulin was measured by a double antibody precipitation method (Morgan and Lazarow, 1963) and sugar was measured in 0.1-ml. aliquots of either blood or plasma by the micro-ferricyanide reduction method on the AutoAnalyzer (Whichelow *et al.*, 1967).

RESULTS

The blood sugar and plasma insulin responses of the two individuals are shown graphically in Figs. 1 and 2. In the woman (Fig. 1) amino-acids alone provoked a rise in the blood

insulin level in the presence of amino-acids was much more dramatic, and the two hormones combined produced an even greater rise in the peak level of plasma insulin.

In the man (Fig. 2) amino-acids alone provoked an initial rise in plasma insulin, the levels then gradually falling during the remainder of the infusion. The effect of secretin and pancreozymin was to maintain insulin levels, raised by amino-acids, at a plateau until the end of the infusion. Secretin and pancreozymin combined, however, produced a very large rise in insulin levels. The two hormones in saline had only a very small effect. It is interesting that this subject (Fig. 2) showed considerable oscillations in the level of plasma insulin with five-minute sampling, a phenomenon which we have observed in lesser degree in other individuals (Boyns *et al.*, 1967) following an insulintropic stimulus.

DISCUSSION

Floyd *et al.* (1966) observed that increases in plasma insulin levels following protein meals could occur despite insignificant increases in plasma amino-nitrogen levels. They suggested that liberation of amino-acids into the gastrointestinal tract during digestion of proteins, or their absorption, might enhance insulin release by a mechanism not mediated directly by the plasma

FIG. 1.—Effect of infusions of amino-acids, pancreozymin, and secretin on the blood (or plasma) sugar and immunoreactive insulin levels in a 34-year old woman. The zero points are the mean of three resting measurements. (AA=amino-acids; SAL=saline; PZ=pancreozymin; SEC=secretin).

FIG. 2.—As for Fig. 1, in a 22-year-old man.

sugar and plasma insulin levels. The latter fell after 20 minutes of the infusion to fasting levels. Pancreozymin given in saline had no detectable effects on either blood sugar or plasma insulin levels, but when given during the second half of an amino-acid infusion the initial rise in plasma insulin continued until the infusion was stopped. The effect of secretin on the plasma

concentration of the absorbed amino-acids. It was earlier shown (Wang and Grossman, 1951) that protein digests introduced into the small intestine of the dog were potent stimulants of the release of both secretin and pancreozymin, hormones which have since been shown to be capable of stimulating the endocrine as well as the exocrine pancreas (Dupré *et al.*, 1966 ;

Boyns *et al.*, 1967; Jarrett and Cohen, 1968). It is conceivable, therefore, that the "enhancement" postulated by Floyd *et al.* (1966) is provided by the action of these two hormones. Turner (1968) has already shown *in vitro* that the insulinotropic effect of the amino-acid leucine is augmented by pancreozymin, while Ohneda *et al.* (1968) have recently reported that pancreozymin augments the insulinotropic effect of amino-acids in the dog. Further support for this hypothesis is provided by the present study, in which an increased insulinotropic effect of secretin and pancreozymin occurred in the presence of raised levels of circulating amino-acids.

Circumstantial evidence for a significant role of secretin and pancreozymin in the insulin response to protein, but not in response to glucose, may be adduced from the work of Estrich *et al.* (1967). These workers observed the effects of several oral test loads and could find no difference between the mean plasma insulin and glucose response to carbohydrate alone or to carbohydrate plus fat, yet fat is also known to stimulate secretin and pancreozymin release (Wang and Grossman, 1951). Carbohydrate plus protein, however, was associated with a significantly greater *insulin* but a lesser *glucose* response than a carbohydrate load alone, suggesting that the augmenting effect of intestinal hormones on insulin release occurs only in the presence of raised levels of plasma amino-acids. This interpretation of the role of these hormones is consistent with the failure to observe any effects of endogenous secretin and pancreozymin on *glucose*-induced insulin release, when intraduodenal hydrochloric acid at physiological concentration was used as the secretory stimulus (Boyns *et al.*, 1967; Mahler and Weisberg, 1968) and with the failure of intraduodenal infu-

sions of glucose to affect *external* pancreatic secretion (Sum and Preshaw, 1967).

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Medical Memoranda

Sustained Azathioprine-induced Remission in Wegener's Granulomatosis

British Medical Journal, 1969, 4, 600-601

Malignant granulomatous disease of the respiratory tract has a relentless progressive course which as yet has not been greatly influenced by treatment.

In the case reported below azathioprine appears to have effected amelioration of this condition. Of particular significance is the continuance of this satisfactory state in the months following cessation of immunosuppressive therapy.

CASE REPORT

In 1962 the patient, a woman then aged 57, had been under investigation because of vitreous opacities. Special examinations showed negative x-ray films of the chest, sinuses, and jaws, and a normal blood urea. No significant features developed until the second half of 1967, when her general health and energy deteriorated. She had lost considerable weight and experienced severe headaches and pronounced nasal obstructive symptoms with discharge, which was not infrequently bloodstained.

On examination the essential abnormality was numerous scattered granulating areas in the nasal, pharyngeal, and subglottic zones. Again x-ray films of the chest, sinuses, and skull showed no changes. Biopsy specimens taken from various affected sites revealed a granuloma, showing dense infiltration with plasma cells, lymphocytes, eosinophils, and multinucleated giant cells, and a vasculitis, consistent with a diagnosis of Wegener's granulomatosis. The patient's general condition indicated considerable ill-health. There was no abnormality of the levels of haemoglobin, white cells (total and differential), platelets, blood urea, or serum protein studies, but the E.S.R. (Westergren) was 106 mm. in the first hour.

Prednisone was prescribed in a dosage of 20 mg. q.d.s. No response in her local or general state resulted, and while under treatment she complained of numbness and paraesthesia of the distal parts of the lower limbs. Abnormal neurological findings were hypoaesthesia to the level just above the ankles, with impairment of proprioceptive sensibility and an ataxic gait, but no motor phenomena were evident. In view of the progress of the disease in spite of corticosteroid treatment, this was gradually "tailed off," and azathioprine was prescribed at a daily dosage of 100 mg. Within a few days the patient had improved subjectively and objectively with the disappearance of the neurological signs of the polyneuropathy and the granulomata, so that after two months on azathioprine therapy no lesions were visible in the upper respiratory tract.

After one month's treatment the E.S.R. reading in the first hour fell to 36 mm. and subsequently was maintained in the region of 10 mm.

The patient was discharged on a dosage of 50 mg. of azathioprine daily, but several weeks later anorexia and nausea appeared, and as these effects were attributed to drug intolerance azathioprine was discontinued. To date the general well-being has been maintained, regular re-examinations have shown no indication of local recurrence of the granulomatous condition, and the E.S.R. level has not increased.

In summary, her prednisone therapy extended over three and a half weeks before it was progressively reduced; azathioprine was then prescribed at 100 mg. daily for two months and at 50 mg. daily for a further 10 weeks. All therapy has been discontinued over a 15-month period, with no indication of recrudescence of the disease process.

COMMENT

The concept that Wegener's granulomatosis represents an immunological reaction prompted the use of corticosteroid therapy. Reports of the results of such treatment have been somewhat contradictory, but in general it has proved of limited value and has been attended by multiple side-effects (Beidelman, 1963).