Preliminary Communications

INTRAPORTAL INSULIN PROTECTS FROM THE LIVER INJURY OF PORTACAVAL SHUNT IN DOGS

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Summary 4 days after portacaval shunt, the livers of normal dogs had pronounced atrophy and other structural abnormalities. These changes were greatly reduced in the left liver lobes, but not in the right, by a constant infusion to the left portal vein of insulin in non-hypoglycaemic doses. These experimental findings should have implications in clinical medicine.

INTRODUCTION

During the past ten years we have developed evidence that substances, termed hepatotropic factors, in the portal venous blood of dogs can profoundly influence liver function as well as the size, internal structure, chemical composition, and dividing capability of the liver function as well as the size, internal structure, chemical composition, and dividing capability of the liver itself.1-3 In most of these experiments, techniques were exploited that permitted comparative study of two portions of the same liver which were given different kinds of portal venous inflow under diabetic or non-diabetic conditions. The concept emerged that manifold hepatotropic constituents are delivered straight to the liver, with a presumably augmented significance because of the episodically high concentrations of nutrient substrate in the same blood.2-4 Insulin has been identified as the most important of these undoubtedly multiple portal hepatotropic constituents.5-9

If the foregoing conclusions were correct, the aetiology of the well-recognised but poorly understood hepatic injury caused by portacaval shunt in animals should become explicable. Furthermore, it should be possible to minimise this damage by infusing insulin into the liver from which portal flow has been diverted. We have performed this experiment in dogs with results that should have clinical implications.

METHODS

Eight normal mongrel dogs averaging about 19 kg had large side-to-side portacaval shunts (group 1). The shunts were made completely diverting by ligating the right and left portal branches at their origin. Thirteen more dogs of approximately the same weight had the same procedure except that the tip of a fine infusion catheter was placed into the tied-off left portal branch and led outside to a small finger pump that was incorporated into a light body cast. A pump infusion of regular insulin diluted in heparinised physiological saline was started, using volumes that never exceeded 21 ml/day. The thirteen test dogs were divided into two subgroups of nine (group 2) and four (group 3) on the basis of the insulin dose that was given (see accompanying table). Ten normal dogs in our laboratory had morning blood-sugar concentrations that were never more than 210 mg/dl, and four dogs treated with low insulin doses had 13 sugar determinations that were 71-2-8-4 mg/dl. All dogs were on an ad-libitum diet from the 1st post-operative day onward.

The experiments lasted 4 days. Their design permitted an evaluation of any direct protective effect of insulin upon the left lobar hepatic tissues as well as a judgment whether insulin which passed through the left lobes without being consumed or degraded had a spillover effect upon the right side after recirculation. Histopathological end-points were used. The size of the hepatocytes was determined on haematoxylin-and-eosin-stained sections by a method previously described.2 In essence, the technique consists in tracing out large numbers of hepatocytes on standard-thickness paper, cutting out the silhouettes, and weighing them. The weights were designated as size units. Methods for other light and electron microscopic examinations that were made in selected experiments were as described before.2-4

RESULTS

The insulin infusions into the left portal vein reduced markedly but did not completely prevent atrophy in the left lobes (table and figure). The protective effect occurred with both
higher and lower insulin dose ranges, but somewhat less with the low doses. There was no spillover protection whatever for the right lobes (table). The differences in the day-4 left lobar biopsies between all thirteen treated dogs versus the eight controls were significant (p < 0.001) as were the differences between the day-4 left-versus-right lobar biopsies in the pooled thirteen treated animals (p < 0.001).

The maintenance of the size of the hepatocytes in the left lobes of the animals treated with insulin was not due to an accumulation of fat or glycogen in the cytoplasm (figure). This was confirmed by electron microscopy which showed that these hepatocytes had an essentially normal ultrastructure. In contrast, the atrophic right-lobate hepatocytes showed an increased number of small lipid vacuoles and other inclusion bodies, reduction in amount and dilatation of the rough endoplasmic reticulum, and swelling of the mitochondria.

DISCUSSION

These experiments were planned after it was realised in earlier work with what remarkable rapidity liver tissue in dogs was altered if it was denied exposure to either total splanchnic venous blood or that portion of it which returns from the pancreas. Thus, it was not surprising that in untreated control animals hepatocyte atrophy and other morphological changes within 4 days after portacaval shunt were almost as pronounced as those we have seen 2 months after this operation. Nor was it unexpected that intraportal insulin in non-hyperglycemic doses sharply reduced these structural abnormalities in the liver lobes directly exposed to the infusion, but not in the contralateral lobes—presumably because the liver avidly removes insulin on first pass.

The failure of insulin to completely protect even the directly infused liver probably means that other individually less important substances in splanchnic blood have a significant composite, hepatotrophic effect as we have suggested in the past from other lines of evidence.2,3

The concept of an interplay between the liver and the other splanchnic organs in which the liver is the target and not just the monitor and integrator of hormonal messages has many specific implications apart from a broader understanding of hepatic physiology. It has already permitted an approach to such diverse clinical issues as the correct revascularisation of liver homografts;1 the means by which portal diversion can benefit patients with hyperlipidemia;4 and glycogen-storage disease,4 the mechanism by which portal blood affects liver regeneration;5,6 and the proper selection of shunt procedures to decompress oesophageal varices.

The observations of this report hold open two additional possibilities of investigation. First the therapeutic effect of intraportal insulin alone and in combination with other possible collaborating hepatotrophic substances (such as glucagon) should be investigated in appropriate animal models of acute liver injury other than the Eck fistula preparation which we used. It would be surprising if the course of recovery from other kinds of injury could not be influenced. Second, the same approach of hormone therapy should be evaluated in an attack on the root cause of the hepatic encephalopathy that can occur in animals and man with liver failure or after completely diverting portacaval shunt. Past efforts have tended to focus on the toxic products causing the encephalopathic syndromes rather than upon the organ whose malfunction is responsible.

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REFERENCES


IMMUNOTHERAPY OF NON-CLINICAL VAGINAL CANCER

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Summary In six women who had had a hysterectomy and three or more vaginal smears positive for malignant cells but no symptoms, a course of immunotherapy, based on delayed hypersensitivity reaction to dinitrochlorobenzene, was tried. Repeat smears done every three months have all been normal, and the patients were well when last seen 2–35 months after treatment.

INTRODUCTION

In patients who have had a hysterectomy, the appearance of malignant cells in a vaginal smear without clinical evidence of cancer presents a problem. Skilled colposcopic examination of the vagina may demonstrate the site of the microscopic lesion, allowing local surgery to be done, but usually, in the absence of a colposcope, the whole vagina is removed with or without vaginal replacement, depending upon the patient's sexual needs.

In 1968, Klein described a method of immunotherapy of cutaneous and mucosal neoplasms1 based on the delayed-hypersensitivity response to triaziquone ("Trenimon"). Following correspondence with Dr Klein, we have used the delayed-hypersensitivity response to D.N.C.B. (dinitrochlorobenzene) in an attempt to treat non-clinical vaginal cancer.
PATIENTS AND METHODS

Patients
All seven patients had had a hysterectomy, and before D.N.C.B. treatment all had at least three vaginal smears showing the presence of malignant cells, confirmed by at least two independent cytologists. Full examination of the vagina did not reveal any evidence of invasive cancer or any other suspicious lesion.

Methods
Patients were given a tube of 0.1% D.N.C.B. in simple aqueous cream base, and told to apply a small amount of cream at least once daily inside a circle 1 cm in diameter drawn on either an arm or leg, and to ensure that there was always some cream in contact with their skin at that point. No dressing was applied. The patients were told to expect a reaction to the cream at the site of application after 3 or 4 weeks; this reaction usually consisting of some redness of the skin and local irritation although occasionally blistering occurred. Patients were told to stop applying the cream as soon as they got a reaction. The degree of sensitivity was then assessed by applying four different concentrations of D.N.C.B. (0.1%, 0.05%, 0.005%, and 0.0005%) in four adjacent areas of the skin, twice in a period of 48 h. The patch tests were covered by a simple dressing. After 48 h that concentration of D.N.C.B. which only just produced a reaction was chosen and used for local application to the vagina.

The vulval area was liberally coated with sterile white paraffin, in an attempt to prevent local reaction on the vulva from vaginal spillage. Using a 20 ml syringe and a firm plastic tube, 14 ml of the chosen concentration of D.N.C.B. in simple cream was inserted at the vaginal vault. This cream was then distributed over the whole vaginal surface by digital means using a surgical glove. Applications were made daily until there was intense local reaction in the vagina or for 3 successive days, whichever came earlier. The patient was nursed in bed and, after using a bedpan or on any occasion when it was felt there may be spillage of D.N.C.B. onto the vulva, the vulval area was swabbed and white paraffin was liberally reapplied. 24 h after treatment was completed the patient was allowed up, and, provided she could cope with vulval swellings, she was discharged. Each patient was advised that for 1 week after leaving hospital she should not go swimming or sit in a bath in case water entered the vagina. Patients were advised to abstain from sexual activity until they were seen again for repeat cytology 6 weeks later. Cytological examination was then repeated every 3 months for the first 2 years and then every 6 months thereafter.

RESULTS
Of seven patients, one did not become sensitised to D.N.C.B. even after continuous daily application for 5 months, and this method of treatment was abandoned in her case.

The remaining six patients all had normal smears at follow-up and they have remained normal since. Individual follow-up has been for 35, 27, 24, 17, 13, and 2 months so far.

No side-effects have been noted as a result of this treatment, other than some vulval discomfort, as the white paraffin is not completely effective in preventing reaction on the vulva.

DISCUSSION
Not all intraepithelial carcinomas progress to invasive clinical cancer—indeed a small percentage of such lesions may regress completely, such regression probably being immunological in origin. It is presumed that the local infiltration of the vagina by sensitised lymphocytes in the treated patients is similar to that which occurs naturally in those patients where the lesion regresses spontaneously. We believe that the treatment we have described should be applied to any patient with a non-clinical vaginal malignancy, surgery being undertaken only if immunotherapy fails.

We have also treated many patients with carcinoma-in-situ of the cervix by a very similar method. The results are not so straightforward as with non-clinical vaginal cancer, however, and seem to depend on the amount of cervical mucus present and on the exact site of the lesion. Further work is required before we feel able to publish results of immunotherapy for this much more common condition.

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REFERENCE

Hypothesis

FUNCTIONAL SUBDIVISION OF ISLETS OF LANGERHANS AND POSSIBLE ROLE OF D CELLS

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Summary Immunocytochemical examination of the islets of Langerhans in various animal species, including man, indicates that insulin-producing cells (B cells), glucagon-producing cells (A cells), and cells producing somatostatin or a somatostatin-like peptide (D cells) are not randomly arranged within the islet. Whenever A cells are found in the islet—i.e., mostly in its peripheral part—they are accompanied by D cells. However, most B cells, which occupy a central position, are in contact only with other B cells. In view of the inhibitory effect of somatostatin on both insulin and glucagon secretion, it is suggested that the arrangement of A, B and D cells is important to the normal and pathological functioning of the islet.

The islets of Langerhans are clusters of endocrine cells which respond as single functional units to various physiological influences. The recent demonstration of junctional complexes between adjacent islet cells1 has raised the possibility that cell-to-cell communication may help to coordinate their hormonal outputs. We propose, therefore, the concept, based entirely on anatomical findings, that the islet of Langerhans may be functionally divided into two subunits—one, a peripheral heterocellular unit, composed of A, B, and D cells, in which somatostatin release from D cells can inhibit glucagon and insulin secretion from the neighbouring A and B cells; the other, a centrally located homocellular unit, composed mostly of B cells.

The discovery that somatostatin,2 a powerful inhibitor of both insulin and glucagon secretion,1,4 or a soma...
led us to re-evaluate by immunofluorescence techniques the arrangements of insulin, glucagon, and somatostatin containing cells. A most striking feature in all species examined was the parallel distribution of A and D cells, which seemed far too consistent to be a coincidence. Indeed, in the rat, Chinese hamster, and mouse, in which the glucagon-producing A cells occupied the most peripheral area of the islets, the D cells formed a sparser layer in close proximity to the outer A-cell sheath, and peripheral to the great mass of the centrally located islet cells composed mostly of B cells (see accompanying figure). In species, such as the horse, in which the A cells were concentrated in the centre of the islets, the D cells also were more centrally distributed. Finally, in addition to their generally peripheral location, A and D cells tended to be grouped together against capillary walls as clearly delineated cell cords within the islets.

It follows that the percentage of the total A-cell mass in contact with D cells must be greater than the percentage of the B-cell mass, most of which has no D-cell contacts. Consequently, if islet secretion functions as a local inhibitor of neighbouring A and B cells, it would have a greater influence upon pancreatic glucagon secretion than on insulin secretion. Similarly, glucagon could have a greater effect than insulin upon pancreatic somatostatin secretion. Only those B cells in or near the heterocellular rim of the islet containing A, D, and B cells would be affected by locally secreted somatostatin. Could there, then, be two anatomical and functional subunits of the islets of Langerhans, a heterocellular (A, B and D cell) "cortex", where vascular and neural elements abound and D cells are prominent, and a predominantly central region, consisting mainly of B cells? The heterocellular region of the islets of Langerhans could be the acutely responsive dynamic component which participates in the rapid moment-to-moment changes in insulin and glucagon release. The so-called first phase of insulin release, a sudden but short-lived burst, could originate in this region and be quickly quenched by somatostatin release. Inhibition of glucagon secretion by insulin and of insulin secretion by insulin could be mediated by D cells. The central homocellular B-cell mass, by contrast, could be a less dynamic, but steadier, site of insulin production, carrying the burden of the continuing basal and anabolic hormone requirements.

In addition to their anatomical proximity, a quantitative relationship between A and D cells appears to exist. Whenever the A cells were found in great abundance—e.g., in "dark islets" of birds,1 in human juvenile diabetes,2 21 and in streptozotocin diabetic rats17 21—D cells were correspondingly abundant. In the only glucagon-secreting tumour thus far studied in this manner, a considerable increase in somatostatin-staining D cells was found exceeding even the adjacent A cells.21 So far, somatostatin-containing cells have been found in the absence of glucagon-containing cells only in the canine uncinate process.21 The correlation between A and D cell numbers accords with the postulated inhibitory action of D cells upon A-cell secretion. For instance, the intense D-cell hyperplasia observed in some diabetic states, and in a glucagon-secreting tumour could reflect a compensatory response of the D cells, however ineffective, to reduce excessive glucagon release caused respectively by insulin lack or autonomous function by malignant A cells. Although the possibility that insular somatostatin does not leave the islets will make this hypothesis difficult to test, these concepts warrant serious consideration in physiological and pathophysiological issues involving the islets of Langerhans, and a possible role of the D cell as a mediator of negative effects on glucagon and insulin secretion should now be entertained.

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A CELLS Glucagon
D CELLS Somatostatin
B CELLS Insulin

Schematic representation of an islet of Langerhans showing distribution of glucagon, somatostatin, and insulin containing cells.

Islet-cell types for which no positive function has yet been established are omitted.
Insulin and the Liver

The first major impact of the pancreatic hormones, insulin and glucagon, is on the liver through the portal system. Why should pancreas and liver be so closely related anatomically? The liver destroys half or more of the insulin in portal blood, so it obviously has a degradative function, and insulin may also be lost in the bile. Insulin concentrations bathing liver are some two to four times those reaching the periphery, and this observation led to the view that the relation of pancreas to liver has a major regulatory influence on intermediary metabolism. In particular, the relation will be important in the disposal of ingested fuels reaching the liver via the portal route. More recently the simple view that insulin alone determines the way that the liver disposes of circulating fuels has been replaced by a concept giving prime importance to glucagon relative to insulin/glucagon ratio. Experiments by Parilla and his colleagues showed no difference in the effects of insulin and glucagon over a wide concentration range provided that the ratio of the two hormones was kept constant. Unoe has also shown that the glucagon/insulin ratio changes according to whether the organism requires the liver to produce or to conserve fuel, although the importance of glucagon relative to insulin in diabetes mellitus may have been overstated.

An entirely different role of insulin has now been revealed. Over the past decade workers in various laboratories have sought the nature of elusive "hepatotropic" factors which are present in portal venous blood. These factors seem to affect many aspects of the hepatocyte including regeneration, cell size and structure, and lipid metabolism. The presence of hepatotropic factors was suggested 55 years ago by Rous and Larimore but it is only lately that, with the aid of ingenious surgical techniques, suggestions have been made as to their nature. Much of the newer work has come from Marchioro, Porter, Putnam, Starzl, and their colleagues, and it stemmed from studies on the revascularisation of auxiliary liver grafts. Two parts of the same liver were maintained with portal venous inflow of splanchnic or systemic blood. The part of the liver perfused with splanchnic blood showed strikingly greater hyperplasia, hypertrophy, and glycogenation. Subsequent experiments produced hepatocyte atrophy in dogs after portacaval transposition in which lower limb and renal venous blood was diverted to the liver. This neatly negated the suggestion that the hepatic atrophy seen after portacaval shunting is due to decreased flow and strongly supported the role of some chemical factor or factors. In other experiments one part of the liver was supplied by blood primarily from the lower splanchnic bed while the other part of the liver received blood from the pancreatico-gastroduodenal bed. There were dramatic differences between the two parts of the liver. That part receiving pancreaticoduodenal blood showed obvious hypertrophy and hyperplasia as early as four days after the operation while the lobe receiving lower splanchnic blood showed pronounced atrophy, although this was not as severe as in the portal-transposition experiments. Again, flow was not a limiting factor. This led to the suggestion that the pancreatic hormones insulin and glucagon were all-important, with some smaller contribution from lower splanchnic blood due presumably to its high substrate content. However, when alloxan-diabetic dogs were used the beneficial effects of pancreatic blood were lost, despite the presence of glucagon, and no difference was found between pancreactectomised and alloxan-diabetic animals. Suggestions to the contrary notwithstanding, this seemed to eliminate glucagon and left insulin as the probable major factor.

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Dr Starzl and his colleagues in this issue (p. 1241) now provide direct evidence that insulin is indeed a major factor in maintaining hepatic integrity in vivo. In their delightfully simple experiments they confirm first that portacaval shunts in dogs lead to hepatic atrophy. They have then repeated these experiments but infused a small amount of insulin (insufficient to cause hypoglycaemia) into the left portal vein and observed a decrease in hepatocyte shrinkage which could not be accounted for by glycogen deposition. This, the preceding work by Starzl and his colleagues, and previous in-vitro work26 suggest strongly that insulin may have a crucial role in maintenance of hepatocyte integrity and also in hepatic regeneration, although it would be rash to suggest that other factors are not involved as well.

This work, if confirmed in man, has distinct clinical implications. Starzl points out some of these, including the possible value of intraportal insulin in acute liver injury. He makes the intriguing suggestion that the hepatic encephalopathy which follows portacaval shunting or severe liver shrinkage which could not be accounted for by glycogen deposition. This work, if confirmed in man, has distinct implications for our understanding of the pathophysiology of liver disease. It also raises the possibility that insulin may have a role in the treatment of acute cerebral infarction.

Further work is obviously needed to establish the effects on the hepatocyte of systemic insulin, but they are unlikely to be beneficial. A comparison in diabetic animals of the effects on the hepatocyte of islets transplanted into the portal system versus islets transplanted into the extraportal system should provide an answer. Starzl's results should provide another spur to those seeking "physiological" replacement of insulin in diabetes. We should also perhaps look afresh at the use of portal-shunt operations for metabolic diseases such as glycogen storage disease31 32 and intractable familial hypercholesterolemia.33 The operation may be life-saving, in part at least because of diversion of insulin, but it should perhaps be reserved for potentially fatal cases in view of the long-term hepatic atrophy that may follow, owing to that selfsame diversion of insulin. As a final thought, it is intriguing that, even with such a hackneyed hormone as insulin, a new role can still be found and a new and convincing explanation given for the close anatomical relation of the pancreas and the liver.

**Glycerol in Acute Cerebral Infarction**

The list of methods which have been tried for the treatment of acute cerebral infarction vies in length with that for multiple sclerosis, reflecting the ineffectiveness of current therapy in both situations. Anticoagulants,34 35 cortisone,36 37 stellate-ganglion block,34 low-molecular-weight dextran,38 40 and prolonged artificial hyperventilation44 have all been tried for acute cerebral infarction with varying and, often conflicting, results. It might be said that disappointment was inevitable because infarction means the death of neurons, so there is nothing to be saved. This view overlooks two important factors. The first is the contribution of cerebral edema to the mortality and morbidity of cerebral infarction. An infarction of appreciable size provokes edema formation in surrounding tissue, causing further harm to the damaged brain. Prevention or control of edema might be

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expected to lessen damage. The second factor is that not all tissue affected by the primary vascular lesion is infarcted; some is ischemic. Hossmann has shown that neurons which have been rendered ischemic for a matter of some hours may recover their ability to produce evoked potentials and to resume metabolic activity. There is therefore a rational basis for further attempts to treat acute cerebral infarction.

The most recent of these employs intravenous infusions of 10% glycerol in glucose or saline daily for four or six days. The claim is made that glycerol not only reduces oedema by acting as a hyperosmolar agent but also improves cerebral metabolism. Cerebral blood-flow is increased, oxygen consumption and carbon dioxide production by the brain are decreased, glucose consumption remains constant, whilst the respiratory quotient for the cerebral hemisphere falls, suggesting that oxidative phosphorylation has been recoupled. Three controlled clinical trials have been reported; in two glycerol therapy was compared with the non-specific supportive measures employed in the management of strokes and in one with dexamethasone. All three used a scoring system which evaluated mentation, orientation, speech, motor, sensory, and reflex activity, and “performance disability”. None showed significant reduction in mortality; all showed an improvement in the neurological evaluation score which in one was limited to the group with the intermediate score. One patient died of haemoglobinuria and renal failure as a result of the glycerol therapy. The results, though indicating the need for further investigations, are not encouraging. Neurological evaluation scores are notoriously difficult to devise. For example, facial weakness, though disfiguring, does not add to a patient’s disability to the same degree as does weakness of the limbs, yet in one of the trials both are rated the same. In none of the trials is there clear evidence that patients who might otherwise have been bed-ridden were able to achieve a wheelchair life, or those who might only have been able to walk with help became independent.

Further trials will be required in which particular effort should be made to distinguish more precisely the type of lesion responsible for the stroke. For example, the clinical picture produced by a large infarction (such as results from occlusion of the main trunk of the middle cerebral artery) and that caused by a small lacunar lesion in the internal capsule are broadly similar, though differing in detail. The former is very liable to be complicated by massive cerebral oedema, the latter not so. Unless these are separated in a clinical trial of an anti-oedema agent the results are likely to be misleading. Differentiation of these two types of lesion should now be possible with the aid of the EM scan. On the assessment side, whilst evaluation of neurological status should continue, there should be separate assessment of functional capacity—bed-bound, chair-bound, dependent walking, self-care, independent—according to one of the scales in common use.

**SENSE OF HUMOUR**

How can sense of humour (S.O.H.) be evaluated, and would its measurement serve medical purposes? The appropriate procedure is clearly to challenge the patient with humorous material (H.M.) and assess the response. Now the preparation and characterisation of H.M. is no easy task, even with the resources of modern expertise. It is vexingly labile material, degenerating entirely on isolation. Consider, for instance, how Bergson’s specification might work. The trousers of the vain, pompous man fall half down. This makes him seem mechanical, and is comic. It happens again: repetition, and comic. They fall completely: the snowball effect, and comic. They go up again of themselves: reversal, and comic. Simultaneously his neighbour’s trousers fall: reciprocal interference, and comic. But somehow at the end of the isolation procedure not much H.M. remains.

Adaptation to H.M. is clearly a nuisance; we cannot challenge the same patient a second time with the same preparation of H.M., since a single repetition can turn H.M. into a chestnut. Thus testing reproducibility of response is hard, and it is not easy to decide, for example, whether S.O.H. shows a circadian rhythm. Would the joke that fails at 2200 succeed at 1000? —we cannot easily tell. Another problem is that the active groups of H.M. alter from year to year. It is common knowledge that Punch is never what it was. And H.M. varies from country to country. Punch has little to export to the New Yorker, and conversely. Bergson in France reported that people tripping up in the street or losing their hats were laughable. Eysenck took the trouble to observe the effect of a few such incidents in London. No one laughed.

Determining the dose/response relation is a source of difficulty. Lesser responses are detected only by applying a sensitive questionnaire to the patient. Grosser responses appear as grins, chuckles, or outright laughter. There are strange potentations when two can share a joke. The procedure may not even be entirely safe; sources of H.M. such as Gerard Hoffnung’s immortal speech to the Oxford Union, still available on record, may for some subjects be so potent as to merit a Government Health Warning, and readers of Oliver Wendell

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