The results on the overnight urine specimens in all the subjects are shown in the figure. The mean overnight 11-hydroxycorticosteroid levels and the standard deviations in the control, obese, and hirsute groups were similar, being 119±47 nmol (43±17 μg), 121±50 nmol (44±18 μg), and 138±55 nmol (50±20 μg) respectively. The highest value of 271 nmol (98 μg) occurred in an obese patient. By contrast, only one of the patients with Cushing's syndrome had a urinary 11-hydroxycorticosteroid excretion of less than 276 nmol (100 μg) during the night. On the night in question her midnight plasma 11-hydroxycorticosteroid was normal at 189 nmol/l (6·8 μg/100 ml), and her overnight urine contained 254 nmol (92 μg); on a subsequent occasion a repeat overnight collection gave a raised value of 389 nmol (141 μg). The diagnosis of bilateral hyperplasia was confirmed at operation. The overnight specimens from all the other patients with Cushing's syndrome contained more than 276 nmol (100 μg) of 11-hydroxycorticosteroids, the highest containing 2663 nmol (965 μg).

Discussion

Our results confirm that there is a circadian rhythm in the urinary excretion of adrenal steroids in normal people, the lowest levels occurring during the night. In patients with Cushing's syndrome this rhythm is not so apparent, and the nocturnal excretion is much higher.[2 3] We therefore thought that patients with adrenocortical overactivity might be identified by their higher overnight urinary excretion of 11-hydroxycorticosteroids, and this was found to be so.

Our data were obtained solely from women because only one man with Cushing's syndrome was admitted during the study. The overnight 11-hydroxycorticosteroid level in this man with an adrenal carcinoma was grossly raised at 5382 nmol (1950 μg). Thus this test is probably applicable to both men and women.

Clinical suspicion of Cushing's syndrome is often aroused by obesity and hirsutism in women, particularly if it is associated with hypertension or diabetes mellitus, but it is not practicable to investigate all such patients in hospital. This simple screening test, requiring only the collection of an overnight urine specimen for 11-hydroxycorticosteroid estimation, has proved useful in selecting those outpatients who require more extensive investigation. Our data indicate that levels over 276 nmol (100 μg) are highly indicative of adrenocortical overactivity and merit further action. Clearly the diagnosis of such a serious disorder cannot be completely excluded by one normal result, but if doubt persists the test can easily be repeated.

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Enhanced HBsAb production in pathogenesis of fulminant viral hepatitis type B

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Summary

The possible importance of humoral immunity in the pathogenesis of fulminant hepatitis was investigated by comparing 17 patients with fulminant hepatitis type B with 20 patients with severe but non-fulminant disease. Hepatitis B surface antigen (HBsAg) was cleared from the serum significantly faster (P <0·001) in those with fulminant hepatitis, and in 41% anti-HBsAg (HBsAb) was detectable by radioimmunoassay (RIA) at presentation. In all 11 sera from patients with fulminant hepatitis that were examined by electron microscopy aggregates of HBsAg and HBsAb were seen. In contrast, HBsAb was never detected by RIA in those with non-fulminant hepatitis, and in only one serum specimen (5%) were aggregates seen on electron microscopy. A significant sex difference between fulminant and non-fulminant hepatitis was observed, 65% of patients with fulminant hepatitis but only 15% of patients with non-fulminant hepatitis being women (P <0·01). An enhanced production of HBsAb in fulminant hepatitis, by leading to free HBsAb in portal blood, may cause an Arthus reaction in the sinusoids of the liver with ensuing ischaemic necrosis of hepatocytes.

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Introduction

The reasons why viral hepatitis may occasionally follow a fulminant course are poorly understood. It may occur more often after type B infections,[1] and factors such as the dose, virulence, and strain of the virus have been incriminated.[2-4] It has been suggested that in the presence of a normal cell-mediated immune response the outcome of an attack of acute hepatitis depends on the number of infected liver cells.[5] Almeida and Waterson,[6] however, in an electron microscopic study, described the presence of large immune complexes characteristic of antibody excess in the serum of one patient who died from fulminant hepatitis and speculated on the patho-

genetic significance of these complexes. Further evidence of overproduction of antibody in fulminant hepatitis has recently been produced by Trepo *et al.*[7]

To investigate this latter possibility further we compared antibody production and clearance of HBsAg from the serum in 17 patients with fulminant hepatitis due to type B infection and in 20 consecutive patients with acute hepatitis type B who were ill enough to be in hospital.

Patients and methods

The 17 patients with fulminant hepatitis all presented in either grade III or grade IV hepatic encephalopathy, using the criteria of Trey and Davidson.[8] Encephalopathy was not present in any of the 20 consecutive patients with severe acute hepatitis who had been admitted mainly because of the depth or persistence of jaundice. Initial studies in both groups were performed five to 19 days after the onset of jaundice.

Tests for hepatitis B surface antigen (HBsAg) were performed by counterelectrophoresis (CEP)[9] and radioimmunoassay (RIA).[10] Positive samples were titred by complement fixation.[11] Antibody to HBsAg (HBsAb) was detected by RIA.[12]

Sera were clarified by spinning for 10 minutes at 10 000 *g* and spun for a further hour at 55 000 *g*. The resulting pellet was washed, resuspended in distilled water, negatively stained with 3% tungstophosphoric acid (pH 6·5), and examined in an AEI Corinth electron microscope.

Results

The two groups were comparable in age. The range was 18-50 years (mean 34·5 years) in those with fulminant hepatitis and 21-67 years (mean 31 years) in the other group. The sex distribution, however, was quite different. Eleven of the 17 patients with fulminant hepatitis were women, in contrast to only three of the 20 patients with severe acute hepatitis ($\chi^2=7{\cdot}65$; $P<0{\cdot}01$). There also seemed to be a difference in the presumed sources of infection (see table). In most patients with fulminant hepatitis the source of infection was unknown, whereas homosexual contact was the commonest possibility in the non-fulminant group. In only four patients with fulminant hepatitis was blood transfusion or parenteral inoculation incriminated.

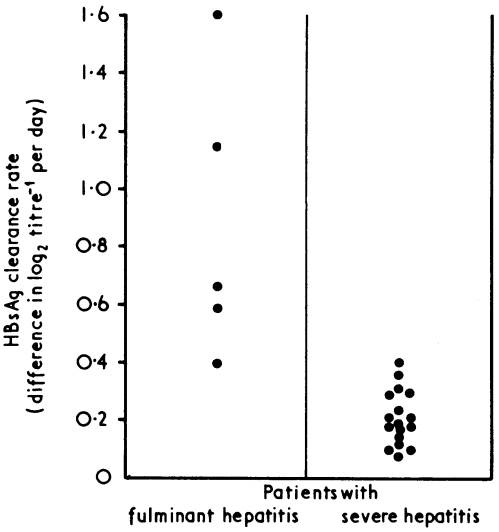
Presumed sources of infection in the two groups of patients

	No with fulminant hepatitis	No with severe hepatitis
Transfusion	2	
Parenteral drug abuse	2	2
Heterosexual contact	1	2
Homosexual contact	1	11
Occupational contact	1	1
Unknown	10	4
Total	17	20

At the time of admission one of the patients was HBsAb-positive by RIA alone, which indicated a low antigen titre. All the others were positive by CEP, and there was no significant difference between the initial titres in the two groups as measured by complement fixation. Sufficient serial titres were available in five of the patients with fulminant hepatitis and 17 of those with severe hepatitis to compare the rates of clearance of HBsAg from the blood in the two groups. This was significantly faster ($t=4{\cdot}04$; $P<0{\cdot}001$) in those with fulminant hepatitis (see figure): the mean duration of antigenaemia was calculated to be 10·7 days compared with 44·1 days in those with severe hepatitis.

A further difference was the finding of HBsAb at admission in seven (41%) of the 17 patients with fulminant hepatitis, whereas this was not present in any of the patients with severe hepatitis ($\chi^2=5{\cdot}46$; $P<0{\cdot}01$) even though most of them had been ill for rather longer before admission to hospital.

Electron microscopy—Sera from 11 of the 17 patients with fulminant hepatitis and from 14 of the 20 patients in the other group were examined by electron microscopy. In all the sera from patients with fulminant hepatitis aggregates of HBsAg and HBsAb were seen, whereas such aggregates were found in only one of the other sera ($\chi^2=8{\cdot}0$; $P<0{\cdot}001$). In some of the serum specimens from patients with fulminant hepatitis Dane particles, as well as small round forms, were seen in the aggregates.



Daily rate of decline in HBsAg titre in 22 patients with viral hepatitis type B.

Discussion

Our findings in patients with fulminant hepatitis agree with those recently reported by Trepo *et al.*[7] The most important finding almost certainly relates to the appearance of HBsAb early in the course of the illness. This is in complete contrast to the more normal form of the disease in which HBsAb is detected only some weeks or more after the onset.[13] As a consequence, HBsAg is cleared very rapidly from the blood in fulminant hepatitis. In the series of Trepo *et al*[7] HBsAg was often present only in low titre and the use of RIA increased the detection rate from 35·9% to 59·3%. In our series, however, the serum was positive by RIA alone in only one patient with fulminant hepatitis, possibly because our patients were investigated earlier in the course of their disease.

It seems unlikely that the development of fulminant hepatitis was related to the dose of inoculum since in only a few cases could a parenteral route of infection be incriminated. "Sporadic" cases of acute hepatitis B have been noted to be of greater severity than those associated with parenteral inoculation, although this difference was attributed to the different antigen subtypes involved.[4]

Massive necrosis of hepatocytes may in some cases be related to the many hepatocytes infected by the virus. Recently we described three patients with blood dyscrasias of various types who acquired HBsAg while receiving immunosuppressive drugs and who developed fulminant hepatitis after this treatment was withdrawn.[14] The most likely explanation seemed to be that during the period of immunosuppression most of the hepatocytes became infected with hepatitis B virus, massive lysis of these cells occurring when the drugs had been stopped and cell-mediated immunity had been restored.

Such considerations do not apply to the present patients, however, in whom the enhanced production of HBsAb may be of considerable pathogenetic importance. Since the spleen is a major site of antibody production,[15] and therefore presumably of HBsAb, HBsAb may be present in excess in portal venous blood. Subsequently it may form complexes in the sinusoids of the liver with HBsAg released from the necrosis of infected hepatocytes by T lymphocytes.[5] The result will be a localised Arthus reaction[16] with ensuing ischaemic necrosis of both infected and uninfected hepatocytes. Only by sampling portal

venous blood directly could such a hypothesis be tested, but are there any therapeutic implications? Unfortunately, once an Arthus reaction has occurred immunosuppressive treatment is unlikely to be of value, which perhaps explains why the use of corticosteroids in fulminant hepatitis has been so disappointing.[17]

The significant female preponderance shown in our patients with fulminant hepatitis may also be relevant. In other conditions associated with hepatitis B infection, such as HBsAg-positive chronic liver disease and the healthy carrier state, as well as uncomplicated acute hepatitis type B, the reverse is true.[18] A female preponderance is known to be present in other conditions, however, such as thyroiditis, systemic lupus erythematosus, and lupoid hepatitis, in which there is an enhanced antibody response to autoantigens, and women may be more prone to this type of immunological hyper-reactivity. Genetic influences may also be involved, as the association of many autoimmune diseases with distinctive HLA phenotypes points to the existence of specific immune response genes in man.[19] Investigation of HLA phenotypes in fulminant hepatitis may provide further evidence to substantiate this possibility.

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Gastric emptying of solid meals in diabetics

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Summary

The gastric emptying rate of an isotopically labelled solid meal was compared in 29 insulin-dependent well-controlled diabetics and 18 normal controls. The diabetics were assessed for evidence of autonomic neuropathy. No significant difference in gastric emptying rate was found between controls and diabetics with or without autonomic neuropathy. Only three diabetics had greatly delayed gastric emptying, but in one of these the test had given a normal result on an earlier occasion.

Introduction

Patients with diabetes mellitus may develop various disturbances of gastrointestinal function. Symptoms include diarrhoea, nausea, and vomiting. Gastric retention as a complication of diabetes was first described by Rundles in 1945.[1] He noted that gastrointestinal symptoms were commonly associated with autonomic neuropathy. In a subsequent study with Hodges and Hanelin he described delayed gastric emptying in five out of 35 diabetics with neuropathy.[2] Martin found delayed gastric emptying in 14 subjects with diabetic diarrhoea.[3]

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The importance of autonomic, and especially vagal nerve, damage in the aetiology of delayed gastric emptying in diabetics has also been discussed by Kassander.[4] He described the condition "gastroparesis diabeticorum" and emphasised that it could occur in entirely asymptomatic subjects.

These and other[5-7] workers have based their observations on the time taken for barium to pass through the stomach. Aylett used liquid meals and an aspiration technique to show that gastric emptying tended to be slower in diabetics than in controls.[8]

Recently it has become possible to measure the rate of emptying of isotopically labelled solid meals by external scanning.[9] We used this technique to compare the rate of gastric emptying in diabetic subjects, with or without autonomic neuropathy, with that in normal controls.

Patients and methods

Twenty-nine insulin-requiring stable diabetics were compared with 18 age- and sex-matched controls. None of the controls had any symptoms of gastrointestinal disease. Neither controls nor diabetics were receiving any medication likely to interfere with gastric function.

GASTRIC EMPTYING MEASUREMENT

Technetium sulphur-colloid (^{99m}Tc) was used as the isotopic marker. Each study used a dose of 250 μCi, which gave a count rate of 20 000 to 50 000 cpm. A gamma-camera was used for external counting.

Subjects fasted overnight and were then given a standard test meal, which consisted of lean meat (beef slices), peas, and rehydrated mashed potato. The total mass of the meal was 250 g, of which the potato weighed 125 g. The isotope was incorporated into the potato mixture by adding it to the hot water used to reconstitute the potato.

Ingestion of the meal took between three and five minutes, after which the diabetics had their usual morning dose of insulin. Each