thyroxine index 8.4. By the eighth month of treatment all parameters of thyroid function had returned to normal, and she remained clinically euthyroid.

Case 4.—Woman aged 57. History of prominence of right eye and gradual weight loss for six months. No other symptoms. Clinically mildly thyrotoxic. Goitre 30 g. Pretreatment 20-minute ^{131}I uptake 12.9%, free thyroxine index 20.5. Remained clinically euthyroid when propranolol stopped after 12 months. Pulse 80/min, regular. Free thyroxine index returned to normal at the 16th month. She remained well and symptom-free.

A similar pattern was observed in a fifth patient in whom the 20-minute ^{131}I uptake fell below 8 by the ninth month of treatment, but the free thyroxine index remained just above the upper limit of normal. The patient was symptom-free and clinically euthyroid, and his weight increased from 64 to 71 kg.

Discussion

While no significant change in the ^{131}I uptake and ^{66}mTc uptake after six months of propranolol therapy had occurred in the group of 21 patients receiving long-term treatment, a definite fall in uptake was observed in individual patients. By six months the disease had remitted in two patients, and at later times all indices of thyroid function had returned to normal in a further two patients. A fifth patient showed evidence of remission with the 20-minute ^{131}I uptake within normal limits, although the free thyroxine index remained slightly raised. It is probable that the decline in thyroid uptake and level of circulating hormone in these patients reflects the natural tendency of the disease to remit, since propranolol is thought not to have any in-vivo effect on thyroid hyperactivity or on the rate of thyroid hormone secretion. There is, therefore, no doubt that prolonged remission may occur during treatment with propranolol. However, since treatment had to be withdrawn in seven patients after one or two months, and since circulating levels of thyroid hormone did not consistently return to normal in the remainder who received the long-term therapy, propranolol cannot be recommended as a definitive form of therapy for the treatment of thyrotoxicosis.

Furthermore, the metabolic consequences of long-term adrenergic blockade in thyrotoxicosis have not been fully

elucidated—for example, the possibility of progressive osteoporosis in some patients exists. Nevertheless it is clear that propranolol has a place in the treatment of thyrotoxicosis in special circumstances (Vinik *et al.*, 1968; Das and Krieger, 1969; Becker and Schwartz, 1972). In this series no significant adverse effects were noted and serum transaminases remained within normal limits. It may be asked if an increase in the dose of propranolol administered might have resulted in a more complete control of symptoms, but the dose administered, 40 mg three times daily, was adequate to consistently restore the pulse rate to normal.

It is not possible from the present study to compare remission rate during treatment with propranolol with that following conventional antithyroid drug therapy, since most workers have given antithyroid drugs for longer periods. It must also be remembered that seven patients had to be excluded from the analysis shown in the table, and patients with severe symptoms were in any case not included in the series. Pimstone *et al.* (1969) observed that in 22% of 27 thyrotoxic patients treated with propranolol alone for a mean period of five months the radioactive uptakes fell to normal, but no estimate of the level of circulating thyroid hormone was reported. We have observed that the radioiodine uptake may return to normal before circulating hormone levels. Our evidence does not support the suggestion that propranolol may be a suitable alternative to treatment with conventional antithyroid drugs in milder cases of thyrotoxicosis.

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Hepatitis B Antigen Inhibitor in Human Faeces and Intestinal Mucosa

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Summary

Hepatitis B antigen (HB Ag) positive sera became negative after in-vitro incubation with homogenates of human faeces or intestinal mucosa. This was found to occur in all sera

tested by various methods. These findings suggest the existence in the human intestine of a substance able to inactivate the HB Ag and that it is not an antibody or of the nature of interferon. The presence of an inhibitor could explain why B-type hepatitis is seldom if ever faecally transmitted and also the low oral infectiousness of the B virus.

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Introduction

B-type serum hepatitis, despite a presumably high concentration of virus in the liver and bile, seems seldom to be faecally transmitted (Krugman *et al.*, 1967; Krugman and

Giles, 1970; Sherlock, 1972; Cossart, 1972). Perhaps the virus is present in the faeces but in a non-infectious state (De Ritis *et al.*, 1959) or it is inactivated by some intestinal agent (Piazza, 1965; 1969). We have studied this interesting problem in mice, using the MHV-3 hepatitis virus (Dick *et al.*, 1956). This virus produces in mice a disease very similar to the one produced by the B virus in man, and its histological, enzymoplasmatic, and pathogenic characteristics are also similar (De Ritis *et al.*, 1957; Piazza, 1969; Piazza *et al.*, 1972). Animals killed at the height of the disease showed high concentrations of virus in the bile and liver, which gradually decreased during passage through the intestine and disappeared completely in the colon and faeces. This suggested that there might be a factor in the mouse intestine capable of inactivating the MHV-3 virus, and this was experimentally proved by finding that MHV-3 virus incubated with homogenate of intestine or of faeces from normal mice lost its capacity to infect (Piazza, 1964).

This factor is perhaps the most active among the known biological inhibitors with antiviral power (a few mg of intestinal tissue of a normal mouse in the proper conditions inactivates 10^7 LD₅₀ of virus). It has been found only in the intestine of various strains of normal and germ-free mice (Swiss, C3H, C57, Balb C) and is present mostly in the mucosa. It inactivates only MHV-3 virus, and is itself inactivated when kept for 10 minutes at a temperature of 80°C (Piazza *et al.*, 1965; Piazza and Salomon, 1966). Its inhibiting power is directly proportional to its concentration. In-vitro studies have shown that at 37°C the inhibiting power increases with the period of incubation. At 2°C the inhibitor is inactive even after four hours' incubation (Piazza *et al.*, 1967).

After the discovery of the hepatitis B antigen (HB Ag) (Blumberg *et al.*, 1967), now generally considered to be the causative agent of B-type hepatitis or, at least, closely related to it (Prince, 1968; Giles *et al.*, 1969; Krugman and Giles, 1970; Sherlock, 1972; Zuckerman, 1972; Wright, 1972), we decided to see whether we could repeat in man the results we had obtained with MHV-3 virus in mice. We had already shown that HB Ag disappeared from 60% of sera incubated with a homogenate of faeces from healthy men, using the method of double diffusion in agarose (Piazza *et al.*, 1971), and we therefore thought it would be of interest to continue our study, using more sensitive methods.

Materials and Methods

Samples of faeces from healthy persons aged from 30 to 40 years and from different animals (dog, cat, horse, rat, cow, sheep, pig, goat, pigeon, mouse, chick) were homogenized separately with normal saline, usually at a dilution of 1/7. In some experiments different dilutions were used. The homogenates were centrifuged at 2,000 r.p.m. for 15 minutes and the supernatant used for testing.

Samples of small intestine and of small-intestinal mucosa from an 80-year-old patient operated on for acute intestinal obstruction and from a 46-year-old patient operated on for

intestinal infarction were also homogenized and the supernatant set aside.

Samples of HB Ag-positive serum of a titre of at least 1/8, measured by immunoelectro-osmopheresis, were obtained from men suffering from acute viral hepatitis.

All samples were stored at -40°C.

We had previously measured the inhibiting capacity of human faeces from healthy men either by immunoelectro-osmopheresis (Pesendorfer *et al.*, 1970; Prince and Burke, 1970) or the complement fixation test (Sever, 1962). Our results are shown in tables I and II. Since then we have always used immunoelectro-osmopheresis (Farmitalia kits) to detect the presence of HB Ag, and our method has been as follows.

TABLE I—Inhibitory Effect of Homogenates of Faeces on 30 HB Ag-positive Sera

Incubation Mixtures	Serum Dilutions						
	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64
Serum + saline	30	30	30	30	15	8	4
Serum + human faeces A	2	0	0	0	0	0	0
Serum + human faeces B	3	0	0	0	0	0	0
Serum + human faeces C	1	0	0	0	0	0	0
Serum + canine faeces	30	30	30	30	14	8	3

Figures refer to the number of sera still found positive by immunoelectro-osmopheresis after incubation with different materials. A, B, C are three different normal subjects.

Various dilutions of HB Ag-positive sera to a dilution of 1/64 were prepared. The presence of HB Ag was then tested for in the following mixtures, which had been incubated at 37°C for 120 minutes and shaken every 20 minutes:

0.2 ml serum or dilutions of serum + 0.2 ml normal saline

0.2 ml serum or dilutions of serum + 0.2 ml of sample material.

0.2 ml of sample material + 0.2 ml normal saline.

With the complement fixation test we used the following mixtures, incubated as above:

0.2 ml undiluted serum + 0.2 normal saline

0.2 ml undiluted serum + 0.2 of sample material

0.2 ml of sample material + 0.2 ml normal saline.

The fixed complement reaction was measured with the Micro-titer apparatus (Cooke Eng. Co., Alexandria, Va., U.S.A.), according to Sever's (1962) methods.

All experiments were done double blind, and the sera and faeces from the healthy subjects were tested against HB Ag-positive sera to detect the presence of HB antibodies.

Results

The results of these experiments (tables I-VIII, figs. 1-3) showed that homogenates of human faeces inhibited the HB Ag in every case, and the findings were the same whether measured by immunodiffusion (Piazza *et al.*, 1971), immuno-

TABLE II—Inhibitory Effect of Homogenate of Faeces on HB Ag-positive Sera Measured by Complement Fixation Test. Results given as HB Ag Titres

S1 + saline = 64	S2 + saline = 256	S3 + saline = 128	S4 + saline = 16	S5 + saline = 512
S1 + F1 = 0	S2 + F1 = 0	S3 + F1 = 0	S4 + F1 = 0	S5 + F1 = 256
S1 + F2 = 0	S2 + F2 = 0	S3 + F2 = 0	S4 + F2 = 0	S5 + F2 = 0
S1 + F3 = 0	S2 + F3 = 0	S3 + F3 = 0	S4 + F3 = 0	S5 + F3 = 0
S1 + F4 = 0	S2 + F4 = 0	S3 + F4 = 0	S4 + F4 = 0	S5 + F4 = 0
S6 + saline = 8	S7 + saline = 16	S8 + saline = 16	S9 + saline = 32	S10 + saline = 32
S6 + F1 = 0	S7 + F1 = 0	S8 + F1 = 0	S9 + F1 = 0	S10 + F1 = 0
S6 + F2 = 0	S7 + F2 = 0	S8 + F2 = 0	S9 + F2 = 0	S10 + F2 = 0
S6 + F3 = 0	S7 + F3 = 0	S8 + F3 = 0	S9 + F3 = 0	S10 + F3 = 0
S6 + F4 = 0	S7 + F4 = 0	S8 + F4 = 0	S9 + F4 = 0	S10 + F4 = 0
	S7 + F5 = 16	S8 + F5 = 16	S9 + F5 = 32	S10 + F5 = 32
	S7 + F6 = 16	S8 + F6 = 16	S9 + F6 = 32	S10 + F6 = 32

S1-S10 = HB Ag-positive sera from 10 different subjects. F1-F4 = Faeces from four healthy subjects. F5, F6 = Faeces from two healthy dogs.

electro-osmopheresis, or complement fixation (tables I and II). Homogenates of human intestine had a similar inhibiting effect on HB Ag and, furthermore, the inhibitor was shown to be present mainly in homogenates of intestinal mucosa (tables III and IV).

TABLE III—*Inhibitory Effect of Homogenate of Intestinal Mucosa and of Intestine Deprived of the Mucosa from an 80-year-old Subject on three HB Ag-positive Sera*

Incubation Mixtures	Serum Dilutions					
	Undiluted	1/2	1/4	1/8	1/16	1/32
Serum + saline ..	3	3	3	3	2	1
Serum + mucosa (1/3 diluted) ..	0	0	0	0	0	0
Serum + mucosa (1/4 diluted) ..	2	1	1	0	0	0
Serum + mucosa (1/10 diluted) ..	3	3	3	1	0	0
Serum + intestine deprived of mucosa (1/4 diluted) ..	3	3	3	2	0	0
Serum + intestine deprived of mucosa (1/10 diluted) ..	3	3	3	3	2	0

Figures refer to number of sera still positive after incubation with different sample materials.

TABLE IV—*Inhibitory Effect of Homogenate of Intestinal Mucosa and Intestine Deprived of Mucosa from a 46-year-old Subject on five HB Ag-positive Sera*

Incubation Mixtures	Serum Dilutions						
	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64
Serum + saline ..	5	5	5	5	5	3	1
Serum + mucosa (1/3 diluted) ..	3	1	0	0	0	0	0
Serum + intestine deprived of mucosa (1/3 diluted) ..	5	5	4	1	0	0	0

Figures refer to the number of sera still positive after incubation with different sample materials.

Except for a slight activity in rat faeces homogenate, only human faeces showed any inhibiting effect on HB Ag. Moreover, the inhibiting effect of rat faeces was the same whether the homogenate was incubated with the serum for five or 120 minutes (table V). Therefore the inhibitory mechanism in rat faeces must differ from that of human faeces (fig. 1).

TABLE V—*Inhibitory Effect of Homogenates of Faeces from Various Animal Species on three HB Ag-positive Sera*

Incubation Mixtures	Serum Dilutions						
	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64
Serum + saline ..	3	3	3	3	3	1	0
Serum + human faeces ..	0	0	0	0	0	0	0
Serum + canine faeces ..	3	3	3	3	3	1	0
Serum + rat faeces (incubated 5 min) ..	3	3	3	3	1	0	0
Serum + rat faeces (incubated 120 min) ..	3	3	3	3	2	0	0
Serum + mouse faeces ..	3	3	3	3	2	0	0
Serum + mouse intestine ..	3	3	3	3	3	0	0
Serum + sheep faeces ..	3	3	3	3	3	1	0
Serum + pig faeces ..	3	3	3	3	2	0	0
Serum + cow faeces ..	3	3	3	3	3	0	0
Serum + goat faeces ..	3	3	3	3	3	0	0
Serum + pigeon faeces ..	3	3	3	3	3	1	0
Serum + chick faeces ..	3	3	3	3	3	2	0
Serum + cat faeces ..	3	3	3	3	2	1	0
Serum + horse faeces ..	3	3	3	3	2	1	0
Serum + donkey faeces ..	3	3	3	3	3	1	0

Figures refer to the number of sera still positive after incubation with different sample materials.

The activity of the inhibitor increased with the period of incubation at a temperature of 37°C. At 2°C no activity was found, whatever the incubation period. Therefore interaction between inhibitor and HB Ag was evidently dependent on temperature and incubation time (fig. 1). Inhibitory activity was also directly proportional to the concentration of homogenate (fig. 2).

The inhibitory activity of crude homogenates decreased when incubated at various temperatures above 40°C for 10

FIG. 1—Interaction between inhibitor and HB Ag at 2°C and at 37°C. Three experiments were performed with similar results. Data from one experiment are shown.

FIG. 2—Relation between homogenate dilution and inhibiting effect. Three experiments were performed with similar results. Data from one experiment are shown.

FIG. 3—Effect of temperature on inhibitor of HB Ag. Three experiments were performed with similar results. Data from one experiment are shown.

minutes and completely disappeared at 100°C (fig. 3), but it was unaffected after 96 hours' dialysis at 2°C (table VI) or after alternate rapid freezing and thawing 12 times (table VII).

Acholic faeces (absence of stercobilin) of patients affected by occlusive jaundice had the same inhibitory activity as those of healthy subjects (table VIII). The gastric juice (at physiological values) of healthy men undiluted or at different dilutions did not show any inhibiting effect (table VIII).

HB antibodies were not found in either the sera or faeces of any of the healthy subjects.

TABLE VI—Effect of Dialysis on HB Ag Inhibitor

Incubation Mixtures	Serum Dilutions						
	Undiluted	1/2	1/4	1/6	1/16	1/32	1/64
Serum + saline	3	3	3	3	3	2	1
Serum + dialysed material 24 hr	0	0	0	0	0	0	0
Serum + dialysed material 48 hr	1	0	0	0	0	0	0
Serum + dialysed material 72 hr	0	0	0	0	0	0	0
Serum + dialysed material 96 hr	0	0	0	0	0	0	0

Three HB Ag-positive sera were used. Figures refer to the number of sera still positive after incubation with different sample materials.

TABLE VII—Effect of Repeated Freezing and Thawing on HB Ag Inhibitor

Incubation Mixtures	Serum Dilutions						
	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64
Serum + saline	3	3	3	3	3	2	0
Serum + frozen and thawed material 2 ×	0	0	0	0	0	0	0
Serum + frozen and thawed material 8 ×	0	0	0	0	0	0	0
Serum + frozen and thawed material 12 ×	0	0	0	0	0	0	0

Three HB Ag-positive sera were used. Figures refer to the number of sera still positive after incubation with different sample materials.

TABLE VIII—Inhibitory Effect of Various Human Materials on three HB Ag-positive Sera

Incubation Mixtures	Serum Dilutions					
	Undiluted	1/2	1/4	1/8	1/16	1/32
Serum + saline	3	3	3	3	2	0
Serum + acholic faeces	0	0	0	0	0	0
Serum + undiluted gastric juice	3	3	3	3	3	0
Serum + gastric juice (1/2 diluted)	3	3	3	3	2	0
Serum + gastric juice (1/4 diluted)	3	3	3	3	3	1

Figures refer to number of sera still positive after incubation with different sample materials.

Discussion

It seems that the faeces of healthy persons contain a substance that neutralizes HB Ag, and that it is produced in the intestinal mucosa and thence passes to the faeces. This could explain the non-infectiousness of faeces of patients with B-type hepatitis.

Some workers have found HB Ag in the faeces in a large proportion of persons with HB Ag-positive serum (Grob and Jemelka, 1971), while other workers have not found it in any (Cossart and Vahrman, 1970; Gust *et al.*, 1971). The presence of HB Ag in the faeces of persons with antigen in the blood would seem to disprove the existence of an intestinal inhibitor, but it could be explained by the amount of inhibitor produced being only small or by the presence of blood in the faeces. Haemorrhoids, for example, are common.

Serpeau *et al.* (1971) found HB Ag in the gall bladder bile but not in the duodenal bile of two people with HB Ag-positive blood. The total bilirubin concentration in the gall bladder bile was two to three times higher than that in the duodenal bile, and it was concluded that the absence of HB Ag from duodenal bile was due to the dilution of the bile in the duodenal juice. We are currently working on this theory, and preliminary findings show that the amount of HB Ag in the gall bladder bile is nearly equal to that in the serum, but in the duodenal bile and faeces we have found no antigen. To discover whether the absence of HB Ag in duodenal bile could be the result of dilution we have made various dilutions of gall bladder bile, and we have found that diluted gall bladder bile contains HB Ag even when the total

bilirubin concentration is one-third of that of duodenal bile. It therefore seems reasonable to think that the absence of HB Ag from duodenal bile could be due not only to dilution but also to the presence of an intestinal inhibitor.

The existence of an intestinal inhibitor could also explain the non-infectiousness of the HB virus by the oral route. In fact the virus present in saliva (Ward *et al.*, 1972), blood, and urine patients with B hepatitis, if introduced by the oral route in normal subjects, could be inactivated by the inhibitor after reaching the intestine. Obviously, however, when there are lesions of the mucosa of the digestive tract, such as an oral, gastric, or duodenal ulcer, the virus can reach the blood stream before reaching the intestine.

Attempts are being made to isolate the inhibitor. In the light of our findings it would seem to be unlike interferon or antibody. The action of interferon implies a cell system in which the virus multiplies, whereas in our experiments the inhibitory action took place *in vitro*. All the human faeces from healthy subjects used in our experiments were tested for the presence of HB antibodies and none were found. Also the inhibitory effect occurred at 37°C but not at 2°C, and this is inconsistent with the nature of antibody. The inhibitor shows interesting similarities to the murine intestinal factor in the mouse which inactivates MHV-3 virus, and we have shown that this is not an antibody (Piazza and Salomon, 1966).

The analogy between experimental mouse hepatitis produced by MHV-3 virus and human hepatitis is interesting. The MHV-3 virus, which is pathogenic only in the mouse, is inactivated by a substance which is present only in the intestine of the mouse. The human B virus—if we assume that it is identical with or at least strictly related to HB Ag—is pathogenic only to man and is inactivated by a substance which is present only in the human intestine. It would seem that the factor which can inhibit the mouse virus and the factor which can inhibit the human virus are not identifiable with any known substances possessing antiviral powers.

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