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Tropical Cirrhosis and Hepatoma

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Those who have ever had to deal, as a clinical or public-health problem, with cirrhosis and primary hepatoma in Third World countries can view with little satisfaction the unresolved dispute about whether their prevalence is to be attributed mainly to nutritional or to infective causes—the one a consequence of natural toxin ingestion, with or without an unbalanced intake of essential food elements, the other declaring itself (as disproportionate HB-antigenaemia percentages among some patient groups) to be the hepatitis B virus infection, with other infections and infestations perhaps contributing. Zuckerman and Dunne (1974) have offered some epidemiological, virological and toxicological reasons why one causative factor need not exclude the other; some animal work may point to a possible additive effect; but for the most part only brief and tentative mentions of possible interrelations find expression in discussions of the aetiology of this widespread health problem.

Some failure in focus may arise in part from use of the word 'Aflatoxin' (AF) itself, in its singular, imprecise, form. Of the several related *Aspergillus flavus* metabolites, Aflatoxin B_1 is the most toxic, and is the liver-affecting fungal metabolite that (with, occasionally, G_1) has been used most commonly in experiments in the last decade. Nevertheless, in nature, several are likely to be encountered together. An imprecision of which this communication is not guilty is the use of the word 'Aflatoxins' as a global term to mean unspecified hepatotoxic fungal metabolites for which there is as yet no generic name (unless the unhandy 'mycohepatotoxins' be allowed). The term 'Aspergillins' proposed by South African workers to include also the toxic metabolites of *A. ochraceus* and *A. fumigatus* (Brown and Abrams, 1965) falls short of meeting the need, for it fails to cover the toxins of *Penicillium, Fusarium,* etc. (Nor ought pyrrolizidines to be called '*Senecio*' alkaloids, for they are found in diverse plant genera (Bull *et al.*, 1968).)

The polarisation of beliefs as to the cause of endemic cirrhosis is nowhere more striking than in respect of the variant Indian Childhood Cirrhosis. Amla *et al.* (1974), submitting powerful evidence for exposure to AF, allow no place for hepatitis B or its antigen (HB Ag); while Chandra (1970), on the basis of a disproportionate occurrence of HB-antigenaemia in his series, considers the disease viral in origin, though conceding that toxic or genetic factors may play some part.

East Africa, on the other hand, first revealed the divergence of the hypotheses in relation to the more widespread adult diseases, cirrhosis and hepatoma. It was in Uganda and Kenya that the surveys of Alpert *et al.* (1971) and Peers and Linsell (1973) related AF consumption to hepatoma incidence; but from Uganda also came the early report (Vogel *et al.*, 1970) of the finding of HB-antigenaemia in hepatoma and cirrhosis oftener than in controls. This, and a subsequent confirmation (Primack *et al.*, 1973), did not propose any monistic view of causation of these conditions; Primack suggests, 'early in life . . . an opportunity for interaction with other common and potentially hepatotoxic environmental factors'; such caution is not always expressed by other 'antigen-protagonists' who have since entered the field.

It is a commonplace that nature when presenting a problem is seldom obliging enough to allow the solitary variable of the well-conducted laboratory experiment. Some suggested interrelations in this problem, with directions of interaction, are set out diagrammatically in Fig. 1 and will be further examined.

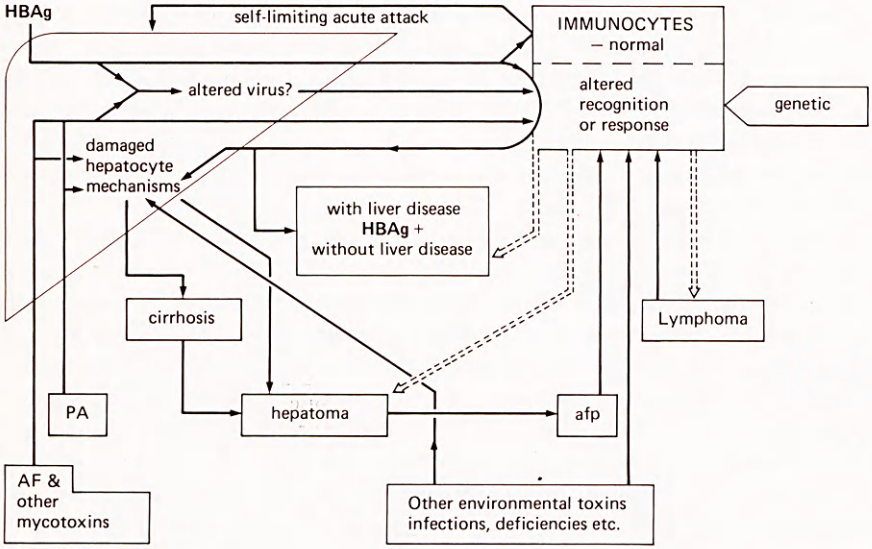

Fig. 1. Possible interrelations of natural toxins and immunological factors in the production of tropical cirrhosis and hepatoma. The broken-line arrows indicate the direction of normal actions that are inhibited.

Evidence for a toxic aetiology of human cirrhosis and hepatoma is largely indirect. Animal models have shown that AF, the most powerful known natural carcinogen, can lead to hepatoma after varying intervals, without any discernible chronic process during such interval (Barnes, 1970). Lafarge and Frayssinet (1970) have offered explanations of the apparent paradox of RNA and DNA

synthesis-inhibition at the nuclear level, with sequential resumption of replication, but a replication restarted with blocked genes and irreversible modifications due to deletions. The acute DNA-blocking effect of AF has been demonstrated in human fetal liver cells by Zuckerman and Dunne (1974) and even non-mammalian eukaryocytes show AF-induced nuclear changes like those in rat liver cells similarly treated (Bauer *et al.,* 1972). Primates, however, respond to repeated small doses by developing cirrhosis, with hepatoma in some survivors (Lin *et al.,* 1974). And while AF is the paradigm hepatotoxic fungal metabolite, *Aspergillus*-derived hepatotoxins like sterygmatocystin and ochratoxin, and the *Penicillium* metabolites luteoskyrin and the chlorine-containing polypeptide (*see* Purchase, 1974) may summate in the long-term exposure of tropical populations, for such fungi may be found together in their stored grain (Coady, 1965).

Experimentally, AF can be shown to interact with another group of naturally-occurring hepatotoxins, the Pyrrolizidine alkaloids (PA) (Newberne and Rogers, 1973). Occurring in disparate plant genera (Bull *et al.,* 1968), these are the likely cause of the well-known 'bush-tea'-induced acute illnesses of Jamaica (veno-occlusive disease—VOD) and of liver disease of chronic evolution due to contamination of grain, as found in South Africa (Willmot and Robertson, 1920), Central Asia (Bronshtein, 1960) and Iraq (Al-Hasany and Mohamed, 1970).

Mattocks (1972) has reviewed laboratory studies on the antimitotic action in the hepatocyte (prefiguring eventual malignancy) of pyrroles, the active metabolites of the alkaloids. Extrapolating from experimental findings, Schoental has invited examination of herbal medicines given to women in pregnancy, in childbirth, or while lactating, or to infants. Appropriately-dosed nursling rats—the alkaloid dose may be single (Schoental and Magee, 1957) or even one delivered via the milk of an unaffected mother (Schoental, 1959)—may develop hepatomas after long periods (sometimes more than two years) of unimpaired growth; the human life-scale equivalent might be many years, as with some industrial toxins. One genus, the leguminous *Crotalarias,* provides potherbs in East Africa, where at least one species so used was found to be hepatotoxic to rats (Schoental and Coady, 1968).

The AF equivalent of the Schoental experiment was recorded by de Iongh *et al.* (1964) who found the milk metabolite 'Aflatoxin M' as acutely toxic to the day-old duckling as Aflatoxin B_1. This important metabolite is produced in the liver in many vertebrate species (Patterson, 1973); Steyn and Purchase (1970) found that female rats tolerated greater doses of AF than male, and that this related to a higher rate of production of the 'M' derivative, which can also be excreted in the urine. They suggested that the liver microsomes in the female, more adapted to processing hormonal steroids, deal more efficiently with the AF nucleus. For this, there is another PA parallel; Tuchweber *et al.* (1974) found that in rats some steroid hormones induced the microsomal enzyme conversion of PAs; the alkaloids monocrotaline and heliotrine were made respectively more and less hepatotoxic. Light is thus shed on the male predominance in most reported series

and also the likelihood (*see* Amla *et al.*, 1974) that the immature organism might begin taking in the toxin literally with its mother's milk.

There is already evidence that both AF and PA may affect immunocytes. Both in clinical and experimental VOD, alkaloid ingestion led to abnormal lymphocyte forms (Martin *et al.*, 1972). AF also impairs *in vitro* human lymphocyte transformation (LCT) in response to phytohaemagglutinin (Aleksandrowicz *et al.*, 1971); partial inhibition of response can be seen in dilutions as low as 0.08 μg/ml (Coady and Brown, 1975). The very organs that gave the two immunocyte categories their names, the thymus and bursa of the chicken, were diminished by AF (Thaxton *et al.*, 1974) to 55 per cent and 30 per cent respectively of control size with doses of 10 μg/g of feed, while an effect on primary immune response was seen with doses as low as 0.625 μg/g. Rats retained labelled AF in a significant amount in the spleen (Lijinsky *et al.*, 1970).

It is reasonable, therefore, to conceive that both hepatocytes and immunocytes may be deleteriously exposed to similar substances simultaneously with hepatocyte colonisation by a virus that the organism cannot eliminate without a fully active immune system. Such impairment of immunity may determine chronicity in infections that might otherwise be dealt with acutely by the cell-mediated immune consequences of virus colonisation; and this may lead to chronic aggressive hepatitis as opposed to acute hepatitis-B, or to immune tolerance without liver symptoms (Dudley *et al.*, 1972; Giustino *et al.*, 1972). These workers predicate three possible outcomes of the invasion by virus B of the liver parenchyma. The virus itself is not directly cytopathogenic; harm only befalls the hepatocyte when the virus modifies its structure so that the altered liver cell is recognised as non-self by the immunocytes (T-cells) and becomes the object of a cell-mediated attack that eliminates the 'tainted' cells in what is, clinically, acute hepatitis. With recovery from this, HB Ag disappears from the blood. If, however, the immunocytes do not recognise the virus-modified hepatocytes as non-self, no such attack and no recognisable disease occurs; the symptomless carrier is produced. A third state ensues when recognition, or response, by the immunocytes is incomplete and an inconclusive, continuing cell-mediated attack induces chronic hepatitis without elimination of antigen; perhaps eventuating in cirrhosis or hepatoma.

It is the two latter possibilities that merit consideration in the attempt to explain the prevalence of HB antigenaemia reported in tropical populations, with and without grave liver disease. It is conceivable that the immune response to the virus might be impaired concurrently with direct chemical, not viral, toxicity to liver cells; a toxicity of itself finding expression, according to species, dose, diet and other determinants, in acute or chronic sequelae. Furthermore, antigen-antibody reactions, in those critical proportions that Almeida and Waterson (1969) consider severally to determine the diverse evolutions of HB infection, must involve both B-cell responsiveness and complement; Notkins (1971) has shown experimentally how complement components interact with virus-antibody

complexes. The Williams group have further examined T- and B-cell interrelations; inclining to discount the importance of circulating immune complexes, they still regard humoral antibody as necessary to clear the antigen, and suggest 'these complex interactions between T- and B-cells initiated by viral infection could be of fundamental importance in the pathogenesis of active chronic hepatitis' (Lee *et al.*, 1975). The surges and wanings of complement levels in acute human hepatitis (Kosmidis and Leader-Williams, 1972), might be influenced by the simultaneous action of AF, since this has been shown to diminish complement in guinea-pigs (Thurston *et al.*, 1972). This could be part of an overall diminution of liver-protein synthesis (Villa-Treviño and Leaver, 1968).

An aspect of HB infection of possible relevance is the increase in antigen titre that precedes clinically patent acute disease (Gocke and Kavey, 1969) and with which a wave of DNA-polymerase activity coincides (Kaplan *et al.*, 1973). Associated with the Dane particle, it is probably dependent on a DNA-template mechanism, for it can be inhibited by actinomycin-D (Bradley *et al.*, 1974). The inhibitory binding to DNA of AF in some ways resembles that of actinomycin-D (Clifford and Rees, 1967; Schabort, 1969; Harley *et al.*, 1969). Actinomycin-D itself inhibits immunocyte response in low concentrations (Kaufman, 1971). How far may an AF-primed host environment affect viral replication as well as the pathological response to this? AF is reported to inhibit a bacteriophage in its action on its normal bacterial host (Jemmali, 1969); while Kremen *et al.* (1974), by dosing tissue-cultures with AF, inhibited or degraded genomic RNA production in avian myeloblastosis virus. One may, however, more readily imagine antigen modification than a beneficial antiviral effect in a host coping with the overall effect of the additive invasions.

The beginning of the harmful process might be seen as an interplay of altered immune stimulus and response (Fig. 2); altered, that is, by naturally occurring chemical factors that are directly toxic to the hepatocyte—substances to which populations in tropical environments may be continuously or frequently exposed—acting simultaneously with a hepatotropic live antigen widespread in those communities. Whether the immune impairment is absolute enough to allow circulation of HB Ag without its damaging the liver or whether the impairment is incomplete, thus leading to active chronic hepatitis, cannot be answered so long as we do not know why interval chronic active hepatitis (antigen-positive or antigen-negative) forms so small a part of the spectrum of chronic liver disease in the affected populations, and why the carrier state exists in such relatively high proportions in the populations of regions where those with hepatoma show no significantly higher prevalence of antigenaemia, like Ethiopia (Samuel *et al.*, 1974), as well as those where, like Uganda or Senegambia, the diseased do show such prevalence. The answer to the latter question presumably lies largely in the opportunities for infection: thus, an American group employed in a plasma fractionation plant were studied by Froesner *et al.* (1975); of those processing the plasma, 82 per cent had anti-HB antibody, and 3.3 per cent were symptomless

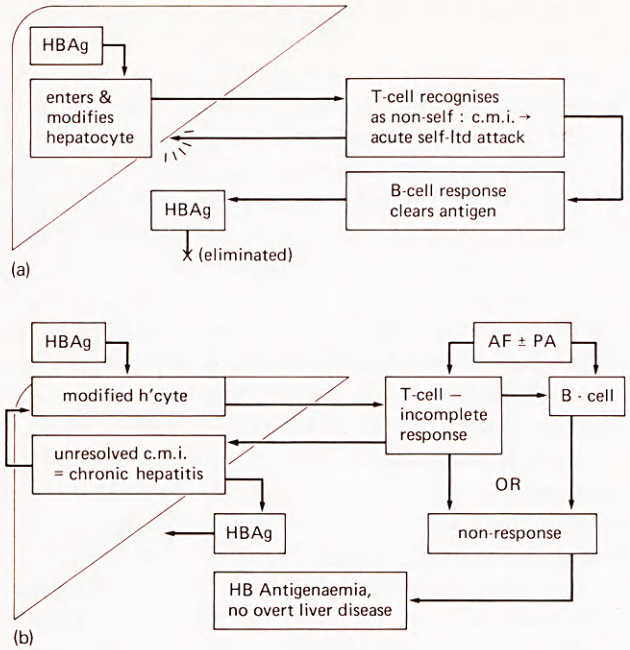

Fig. 2. (a) Normal, and (b) altered consequence of HB virus entry, presupposing simultaneous toxic effect on hepatocyte and immunocyte (and, perhaps also, virion?) leading to unresolved (chronic) c.m.i. process or unrecognised (symptomless) antigenaemia.

antigen carriers, a comparable proportion to those reported from many undeveloped countries; personnel less intimately involved yielded no HB Ag positives.

As part of the structure of the HB antigen is host-derived, Blumberg and Hesser (1975) have proposed that genetically-determined non-recognition of HB Ag may select for survival in undeveloped societies. These societies afford the antigen wide opportunities for spread by traditional practices such as infant head-shaving, group circumcisions, scarification and the like, or transmission by biting arthropods (Prince *et al.*, 1972). If, as outlined above, overt disease presupposes lymphocyte recognition of, and consequent attack on, virus-modified hepatocytes, non-recognition might confer an advantage in terms of morbidity avoided, the negative image of the 'histocompatibility-linked specific immune-response genes' of Benacerraf and McDevitt (McDevitt and Bodmer, 1974).

There are reports of significant predominance of particular HL-A types (HL-A3 and W19) in HB Ag carriers in Belgium (Vermylen *et al.*, 1972); Swiss workers did not confirm this, but noted an inverse prevalence-relationship with the Sabell antigen (Jeannet and Farquet, 1974). In an Australian series, HL-A1 and HL-A8 were over-represented in active chronic hepatitis (HB Ag status unstated) (Mackay

and Morris, 1972). Eddleston and Williams (1974) have suggested how HL-A8 might, by a positive association with defective suppressor T-cells, determine the pattern of immune response to HB Ag in evolving chronic disease. Interestingly, HL-A1 and 8 have also been reported as positively associated with Hodgkin's disease (Honeyman and Menser, 1974), another 'immunoprival' state. The distribution of HL-A types among affected populations in the tropics is little known; but there is an over-representation of lymphomas and (by European standards) precocious Hodgkin's disease in childhood cancers in West Africa (Quénum and N'diaye, 1973), an area of high prevalence of hepatoma and antigenaemia. A somewhat similar lymphoid disease situation with viral associations is reported from East Africa (*Lancet*, 1975). These findings may be relevant in two senses; impaired immune processes seem to be the common factor in the disproportionate HB antigenaemia found with chronic lymphoid abnormalities—the 'Australia affinity group' of the Blumberg school (Sutnick *et al.*, 1972)—while induced immunosuppression (as in transplant patients) may lead, from a presumed hampering of immunocyte surveillance, to the emergence of malignancies, particularly of lymphoid tissues themselves (Hume, 1972; Hoover and Fraumeni, 1973).

There are indications of an association of lymphomas with toxigenic fungi in Eastern Europe (Aleksandrowicz and Smyk, 1973) where 'toxic alimentary aleukia' following the eating of mouldy over-wintered grain was recognised three decades ago, though reported only belatedly in Western-language literature (Joffe, 1974). The toxigenic fusaria implicated there survive low temperatures which preserve the toxic principles; but it is too early to exculpate the traditional tropical grain-storage practices known to favour the toxigenic *Aspergillus* and *Penicillium* (Gilman, 1968; Coady, 1973) from permitting also the production of immunosuppressant metabolites; for instance, *Trichothecium* and *Fusarium* species were found on grains from Ethiopia (Coady, 1965), a country of high prevalence of cirrhosis and hepatoma (Samuel *et al.*, 1974); and trichothecins have been implicated in toxic syndromes with significant haemopoietic disturbance (Saito and Ohtsubo, 1974); some *Fusarium* toxins damage lymphoid tissues (Schoental and Joffe, 1973). The African predominance of childhood lymphomas cited and the significant lymphoma incidence in immunosuppressed transplant patients invites the interpretation that in both groups the 'constabulary' cells had failed, first of all, to suppress the aberrants arising in their own ranks. (To admit a possibility of exogenous influences does not, of course, preclude genetic factors of the kind Buehler *et al.* (1975) have reported from Newfoundland; this group found an extremely high incidence both of immunodeficient states and lymphomas in one inbred family.)

Cancer is currently seen as an expression of failed immunocyte recognition/suppression of deviants from tissue-selfhood (Hellström and Hellström, 1972). Hepatoma may thus result from a runaway mutant arising in a regenerating hepatocyte in cirrhosis (15 to 20 per cent of the cryptogenic cirrhosis in the

western world eventuates in hepatoma); or, in a non-cirrhotic liver, in a cell leached free of DNA-arresting AF or pyrrolizidine to resume disinhibited replication with a consequently damaged template mechanism.

Furthermore, unrestrained liver cell activity can give rise to alpha-fetoprotein (AFP) that may well (Zeigenfuss, 1973) suppress anticancer (and, *a fortiori,* anti-HB Ag) immunosurveillance. The alpha-globulin of normal serum, inhibitory (or 'immunoregulatory') in laboratory-induced LCT (Cooperband *et al.,* 1968), now seems identified as an alpha-macroglobulin (Ford *et al.,* 1973) while a 're-expression of embryonic characteristics' by tumour cells may hinder more specifically the host-protecting cytotoxic response (Rees *et al.,* 1974). AFP positivity, inversely age-related in Bagshawe's (1970) Uganda hepatoma group, can be more widely detected by recent refinements in immunodiagnostic techniques, as in a recent London series (Kohn and Weaver, 1974). The view (*British Medical Journal,* 1970) that AFP production does not accompany experimentally-induced hepatoma is incorrect (*see* Kroes *et al.,* 1972; de Nechaud and Uriel, 1973); it seems justifiable to regard AFP production as liable to arise in any burst of hepatocyte reproduction, malignant or not (Ruoslahti *et al.,* 1974); it is common in Indian childhood cirrhosis sufferers, who do not survive, to get hepatoma (Nayak *et al.,* 1972). Cancer patients in general show a weakening of LCT response, unrelated to the site of the cancer (Kumar and Taylor, 1973), but rather to autologous serum factors. Another liver-derived alpha-protein, alpha-2H-globulin, occurring in malignant disease, has recently been reported to be powerfully immunosuppressant (Buffe and Rimbaut, 1975).

Unlike East Africa and South-East Asia, West Africa as a 'cancer ecology' has not hitherto been systematically studied with respect to mycotoxin exposure of human populations. It is reasonable, following animal work, to envisage protozoal (*Lancet,* 1970) and schistosomal (Warren *et al.,* 1969) depression of the defensive response to viral infection; and Saimot *et al.* (1973) have found, in West Africans working in France, a significantly higher HB Ag carrier-state prevalence (also, of antibodies to *P. malariae*) among those with schistosomal infestation than those free of this: yet these parasites, as well as a differential antigenaemia prevalence, are lacking in upland Ethiopia, where toxin exposure, cirrhosis and widespread hepatoma are recorded (Coady, 1965; Samuel *et al.,* 1974).

Other factors of bacterial origin may affect the liver after reaching it via the gut. Thus, Grant and Roe (1969) found that dimethylbenz(a)-anthracene induced hepatomas much more readily in 'minimal-disease' than in germ-free rats, even though the carcinogen was administered by injection. There are Russian reports (Khanin *et al.,* 1972) that 'dysentery toxin' given simultaneously with 'heliotropium' (i.e. pyrrolizidine) alkaloids in low doses induces cirrhosis in rats. The impairment of the filtering action of the damaged liver may permit antigens from the gut to reach the systemic circulation (Bjørneboe and Prytz, 1972), and provoke hypergammaglobulinemia. Bradfield (1974) has reviewed the role of Kupffer cells in modifying antigen impact; while Broitman *et al.* (1963) offer

experimental evidence that enterogenous toxins from normal gut bacteria can summate with other factors in cirrhosis production.

The diseased liver may have a grossly impaired ability to handle chemical compounds (Adjepon-Yamoah *et al.,* 1974). Liver-microsomal enzymes may be influenced by protein or lipotrope lack (negatively) or inducers (positively) (McLean, 1973) and the enzymes may alter the activity of various hepatotoxins. In rats (Rogers and Newberne, 1971) a diet marginally deficient in lipotropes diminished the acutely-toxic but accelerated the carcinogenic effects of AF. Some pyrrolizidine alkaloids are more, some less toxic to the rat liver after enzyme-induction (Tuchweber *et al.,* 1974). While the effect within the hepatocyte of microsomal handling of AF remains to be clarified (Neal, 1972; Williams and Rabin, 1971), a diet marginal in lipotropes can alter enzyme patterns (Rogers and Newberne, 1971).

In addition to the factors that traditionally fall within the nutritionists' purview, the cation environment may merit closer study; *in vitro* work adumbrates a role for zinc, magnesium and other cations in the LCT response (Berger and Skinner, 1974; Chesters, 1972); the competitive influence of Zn^{++} and Mg^{++} in the binding of AF to DNA (King and Nicholson, 1969) seems relevant. Cell and plasma levels of zinc and magnesium have been found (Rosner and Gorfien, 1968) to be significantly altered in lymphoma and cirrhosis patients. Some of the fungal metabolites may not directly damage the nuclear replicatory mechanisms, but might affect the overall cellular response to those that do; for example, ochratoxin, though no longer thought to be a carcinogen like AF, nevertheless in low concentration inhibits coupled respiration in rat liver mitochondria (Moore and Truelove, 1970).

Virus (or antigen) and ingested toxin interrelations are clearly complex, and we can only begin to guess at the genetic background against which they are played out (*see* Fig. 1) in affected populations. Perhaps, in time, a place will be found for the contribution of other carcinogens, such as the versatile nitrosamine series; or, at the virus polarity, for analogies from the EB virus implication in the natural history of Burkitt's lymphoma. For the present, and while the antigenaemia hypothesis seems to be leading the field, it seems necessary at least to enter a caveat against a premature abandonment of the pursuit of the natural toxins. Confronting the impressive prevalence of HB antigenaemia among some with evident disease, and some without it, we should continue to repeat with Zuckerman the question: is the virus the driver or the passenger?

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Readers will be sorry to learn of Dr Coady's tragic death in an accident soon after the completion of this manuscript, which is a brief memorial of his hard work and deep thought.

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Book Review

Sodium Metabolism in Disease by J. D. Swales. Lloyd-Luke. Price £6

Research in the field of sodium metabolism has expanded very greatly in the past twenty years with the discovery of aldosterone and the elucidation of the renin-angiotensin system. Simultaneously, micropuncture studies have focused attention on renal tubular factors in water and sodium transport. This monograph provides a most welcome summary of this complex subject for the postgraduate student of medicine and, with its 1750 or so references, a most useful guide for those beginning research in the field. The author has naturally had to exercise selection in his choice of references, but he has managed to include much of the important original works as well as very recent studies. The style is clear and readily comprehended by the non-specialist reader. This is not a book for the house officer looking for a quick solution to a practical problem and, indeed, very little space is devoted to management of the various metabolic disturbances.

The interpretation of much of the work referred to is still controversial, particularly in the field of renal tubular sodium and water reabsorption and the existence and role of a natriuretic hormone. A balanced account is given of these and other subjects and the reader is left with an open-mind. A large section is devoted to sodium metabolism in hypertensive states, a field to which the author has made many contributions himself and here, too, he has been careful to present conflicting reports without prejudice.

The book is rather poorly illustrated and what few metabolic studies are presented are restricted to the author's own work.

J.M.L.