time of admission of patients with suspected leukaemia. These were stored at 4° C. until tested within a few hours of collection. Bone-marrow samples were homogenized as much as possible with a pipette and tested without further treatment. Blood samples, which had been collected into equal volumes of Alsever's solution, were centrifuged and the diluted plasma so obtained was tested without further treatment. Both types of sample were inoculated on to Hayflick's mycoplasma medium,[1] but omitting thallous acetate, and also into either semi-solid (0.1% agar) or fluid mycoplasma medium of similar formula. Cultures were inoculated in duplicate and incubated at 37° C. both aerobically and in an atmosphere of 5% CO_2 in nitrogen. At seven and 14 days the semi-solid or fluid cultures were plated on to solid medium and incubated under similar conditions.

The fresh samples were also inoculated into HEp-2 cultures derived from a laboratory sub-line in which mycoplasmas had never been detected. Methods for growth and maintenance of HEp-2 tissue have been described elsewhere.[2,3] The inoculated cell sheets were subcultured after one week by removal from the glass surface with versene, two cultures then being seeded from each original culture. A further subculture was made at the end of the second week. These tissue cultures were tested prior to versenization by plating on solid mycoplasma medium under the conditions outlined above.

The samples tested were from patients with conditions eventually diagnosed as follows: 13 as acute leukaemia, 8 as chronic myeloid leukaemia, 3 as chronic lymphatic leukaemia, 1 as reticulum cell sarcoma, 3 as thrombocythaemia, 1 as polycythaemia, and 1 as splenomegaly. Many of the patients were receiving chemotherapy for their malignant disease at the time of sampling.

No mycoplasmas were isolated in cell-free media in any of the 30 bone-marrow and 30 plasma samples. However, a form of apparent growth was obtained with six of the plasma samples inoculated directly on solid medium. This had the appearance of minute semi-confluent or confluent colonies somewhat resembling those of *Mycoplasma*, but as they could not be subcultured their identity was unconfirmed. The same effect was obtained if penicillin was omitted from the medium. This type of result was obtained with one patient with acute leukaemia, two with chronic myeloid leukaemia, one with chronic lymphatic leukaemia, one with thrombocythaemia, and one with polycythaemia. The same samples inoculated into HEp-2 cultures produced entirely negative results.

On one occasion HEp-2 cultures inoculated with plasma from the patient with a reticulum cell sarcoma were found to be infected with an acid-inducing cytopathic mycoplasma resembling the GDL strain of *M. hyorhinis*.[3-4] The uninoculated control cultures on that occasion appeared to be uninfected. In tests associated with the next sample we isolated a similar mycoplasma, but *only* from the uninoculated HEp-2 culture. Other tissue cultures being handled at this time in the same laboratory were also contaminated with this mycoplasma. It must be presumed that these infections represented chance laboratory contamination, and this example emphasizes the well-recognized risks inherent in the use of tissue cultures as media for isolation of mycoplasmas. We have recently suggested that tissue cultures may sometimes carry crypto-infections of mycoplasmas[2,5] whereby these organisms are present but are not normally detectable by ordinary methods. This hypothesis has to be kept in mind in interpreting results in which mycoplasmas appear to have been isolated by means of tissue cultures rather than cell-free media. However, the susceptibility of tissue culture to mycoplasma infection, already noted, adds significance to our own failure to detect mycoplasmas in any of the HEp-2 cultures inoculated with bone marrow, or in the majority of cultures inoculated with plasma samples.

It is possible that the dubious growth obtained in some of our tests indicates that the medium with which we attempted direct isolation was inadequate, and that more significant results may have been obtained with a suitably modified medium, such as that used successfully by Murphy *et al.*[6] and tried by Dr. Fallon. However, in the absence of further evidence on this point we must interpret as negative the results of our attempts to isolate mycoplasmas from these 30 samples.
—We are, etc.,

London S.W.11. M. BUTLER.
London E.C.1. G. HAMILTON FAIRLEY.
London N.W.9. R. H. LEACH.

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Jarisch–Herxheimer Reaction

SIR,—In your leading article (18 February, p. 384) a plea is made for further research into the nature of the Jarisch–Herxheimer reaction in syphilis. We would like to emphasize that reactions of a similar nature occur in diseases other than syphilis and that a study of this phenomenon on a wider basis might aid in uncovering its mechanism.

In African trypanosomiasis, particularly in the acute *Trypanosoma rhodesiense* infections, pronounced pyrexial reactions to initial treatment are very common in the early stages and still occur, though less frequently, in the later and generally more chronic stages of the disease when central nervous system involvement has developed. In *T. rhodesiense* trypanosomiasis involvement of the central nervous system occurs rapidly and the intermediate stage of the disease may be reached within 4–12 weeks of the onset and the late stage without treatment may be fatal in a few months (3–12). In common with syphilis, the febrile reaction to initial treatment appears more frequently in the early stages of the disease when the trypanosomes are numerous in the blood. It does not appear to matter which anti-trypanosome drug is used, as the febrile reaction is seen with suramin,[1] melarsoprol,[2] and melarsonyl potassium.[3]

In *T. rhodesiense* meningo-encephalitis reactions to arsenical treatment may be very serious indeed.[2,3] In such cases part of the reaction may be due to toxicity of the arsenicals, but the term reactive encephalopathy has been used to indicate the possible pathogenesis as being due to an interaction between the drug, the diseased brain, and the trypanosome. It seems likely that similarities exist between the pathogenesis of such reactions and those recorded in the case of neurosyphilis treated with penicillin.[4,5] The general similarities between syphilis and trypanosomiasis have been discussed over many years.[6]

Whatever the nature of these reactions it is probable that the basic change is similar. Though inpatient facilities for the treatment of syphilis, particularly in the early stage, may not be available, such facilities are essential if close clinical observation of the Jarisch–Herxheimer reaction are to be made. The study of analogous situations in other infections, such as those discussed above, can also be expected to contribute to the eventual elucidation of the reaction.—We are, etc.,

D. H. H. ROBERTSON.
Department of Venereology,
Royal Infirmary of Edinburgh.
W. H. R. LUMSDEN.
Protozoology Research Unit,
Royal (Dick) School of Veterinary Studies,
University of Edinburgh.

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Pressurized Aerosols in Asthma

SIR,—The letter from Dr. M. J. Greenberg and Dr. A. Pines (4 March, p. 563) implicating adrenergic aerosols in the unexplained deterioration and sudden death occasionally seen in asthmatics is one of the more dramatic in a steadily increasing series of unfortunate experiences with these agents. For example, in an early study of the effects of isoprenaline Lowell *et al.*[1] had two deaths in 30 patients with asthma, one occurring quite suddenly at home in a 22-year-old housewife, the second patient dying in status asthmaticus two weeks after he had been placed on isoprenaline aerosols. A causal relationship was not drawn in either instance between the therapeutic aerosol and the development of refractory asthma and death.

At least two mechanisms appear to be involved. Firstly, in susceptible individuals aerosols of isoprenaline given in single or multiple doses may precipitate prolonged asthma, and this phenomenon is reproducible under controlled laboratory conditions at times of the year when the subject is normally in complete remission.[2] Thus when these aerosols are inhaled to counteract bronchospasm due to an extrinsic allergen a summation of challenges rather than protection from the initial antigen challenge may result. In the subjects studied the initial fall in airway resistance following inhalation of the isoprenaline was followed after a period of 1½ to 24 hours by a sustained rise in airway resistance above control. This "boomerang" response is counteracted by the patient with further inhalations, so that increasingly frequent use is made of the nebulizer, resulting in excessive deposition and absorption of the drug. This response is not to be con-