judged by this trial, the 500 µg. dosage provided a reasonable safety margin to allow for irregular taking of therapy.

A similar trial on pregnant patients with established folate deficiency, administering either 500 µg. or 5 mg. folic acid three times daily, also showed no significant difference in response between the two dosage groups. Nevertheless, the need for continued clinical and laboratory supervision needs emphasis, since, even when 15 mg. folic acid is given daily, a small proportion of patients fail to show a satisfactory response.

Could we also draw attention to a minor error in your article? It is stated that oral preparations of folic acid alone in the microgramme range are generally not available. A tablet of folic acid B.P. 100 µg. is now available and is manufactured by McCarthy.—We are, etc.,

B. M. HIBBARD.

ELIZABETH D. HIBBARD.

Department of Obstetrics
and Gynaecology,
The University,
Liverpool 3.

Herpesvirus hominis Infections

SIR,—We noted the letter of Drs. Eleanor J. Bell and Constance A. C. Ross (26 October, p. 254) and find we are in complete agreement with it. We were considering the diagnosis of *Herpesvirus hominis* from the clinicians' point of view on the day of the patient's admission (12 October, p. 89). It was not intended as a comparison of the isolation of the virus on different cell lines; it is well appreciated that the W.I. 38 cells and human amnion are extremely sensitive to *Herpesvirus hominis* infections and may give a typical cytopathic effect in a few days. In our laboratory, the cell lines employed for routine isolation of suspected herpesvirus infections are HEP. 2 and HeLa, though W.I. 38 are used when they are available; the routine use of primary human amnionic cells in some laboratories is not encouraged because their advantages are outweighed by their disadvantages.

A further observation that we should make is that it took, on average, 14.5 days for the virus to be isolated from corneal scrapings as compared with 4.3 days from skin scrapings. This shows that the cell system as regards skins is of a high sensitivity for virus isolation, and that the length of time taken for isolating *Herpesvirus hominis* from eye lesions was solely as a result of the extremely small inoculum, which is fully explained in our paper. Moreover, on the few occasions (not reported) where eye scrapings were used solely for isolation of virus and not for fluorescence as well, the length of time for isolating the virus became as short as that from skin material.

The last point on which we would like to comment is on the question of specificity. Our present reagents do not appear to react with the few cases of florid chicken-pox and herpes zoster that we have examined and have described, material being selected from vesicular lesions. It is possible that with other preparations, cross-reactions may be obtained and in this respect we have so far been fortunate. It is of the utmost importance that fluorescent techniques, for the diagnosis of either *Herpesvirus hominis* or, for that matter, any other virus, should be

specific, accurate, and reliable and acceptable as respectable virus procedures.—We are, etc.,

P. S. GARDNER.

JOYCE MCQUILLIN.

Department of Virology,
Royal Victoria Infirmary,
Newcastle upon Tyne.

SIR,—We write to make two observations on the article by Dr. P. S. Gardner and others (12 October, p. 89).

Firstly, although we have used the immunofluorescent technique to study herpes infections in animals and human brain (as have others before us¹) we do not think it has any advantage for the routine diagnosis of superficial infections. Drs. Eleanor J. Bell and Constance A. C. Ross (26 October, p. 254) commented on the rapidity of virus isolation in human amnion cells, and our results are similar: for 113 isolations, in 1968, of *Herpesvirus hominis* from skin and mouth lesions, the mean time to appearance of characteristic cytopathic effect was 1.7 days (range 1–5). For 63 isolations, in 1965–6, from corneal scrapings the mean time to appearance of cytopathic effect was 1.4 days (range 1–3). When virus can be grown so rapidly fluorescent staining is either just additional labour or a poor alternative to virus isolation.

Secondly, we agree with Dr. Gardner and his colleagues that when rapid diagnosis is really essential immunofluorescence is a useful technique, and we have applied it to 11 biopsy specimens of human brain when herpes encephalitis was suspected and therapy with iododeoxyuridine was being considered. Part of the sample was used for histology, part for virus isolation, and part was blocked in gelatin, sectioned in a cryostat, and stained with fluorescent antibody. We consider sections preferable to smears, because they are uniform and facilitate replication (usually 3-fold) of test and control staining. The specimen from one patient showed equivocal fluorescence, but virus was isolated from it. Specimens from three other patients showed specific fluorescence of infected neurones (5–20 per section) and a strong presumptive diagnosis was made in three hours. Virus was isolated from these specimens one or two days later. Specimens from the other seven patients showed no evidence of herpes virus by immunofluorescence or culture and the patients subsequently proved not to have herpes encephalitis.

Observations on the post-mortem brains of three patients showed that herpes antigen was most easily detected in the cortex, and it is therefore important to include this tissue in the biopsy sample examined.—We are, etc.,

A. H. TOMLINSON.

Public Health Laboratory,
Radcliffe Infirmary,
Oxford.

F. O. MACCALLUM.

Virological Laboratory,
Radcliffe Infirmary,
Oxford.

REFERENCES

- Sabin, A. B., and Messore, G., in *Encephalitis*, edited by L. van Bogaert, J. Radermecker, J. Hozay, and A. Lowenthal, 1961. Amsterdam.
- Johnson, R. T., *J. exp. Med.*, 1964, **119**, 343.

Accidental Poisoning in Childhood

SIR,—Dr. R. H. Jackson and others (26 October, p. 245) underestimate the problem in stating that the incidence of accidental poisoning in children is slowly rising. The Ministry of Health and General Register Office reports on hospital inpatient inquiries¹ show that the number of children admitted to hospitals in England and Wales from the effects of poisons has risen from 4,100 in 1958 to 14,360 in 1965, an increase of three and a half times in seven years. In 1965 there were, on an average, 1,000 hospital beds in daily use for cases of accidental poisoning in children.

Dr. Jackson's figures confirm that the majority of poisons taken are now tablets and not domestic substances, and of the tablets about half are aspirins. It seems probable that between three and four thousand children are admitted to hospital each year with aspirin poisoning. A personal study of 200 such cases over the last four years has shown that the drug has nearly always been taken in the form of junior flavoured aspirin, and that the child practically always finishes the bottle or packet, which usually contains initially 50 tablets (4 g.). A simple preventive measure would be to stop the sale of junior aspirin in packages of more than a dozen (1 g.). This amount of aspirin is unlikely to give rise to toxic effects.

Of the several preventive measures suggested by Dr. Jackson one of the simplest and most practicable would be to insist on the labelling of containers with the name of the drug. This has been advocated by all sections of the medical profession, by the Pharmaceutical Society, and strongly recommended by the Committee on the Safety of Drugs. The time has surely arrived for the Minister to enforce this by regulation.—I am, etc.,

W. P. SWEETNAM.

Department of Paediatrics,
The Royal Infirmary,
Huddersfield, Yorks.

REFERENCE

- Report on Hospital In-Patient Enquiry for the year 1965, Part I, Tables, H.M.S.O., London, 1968.

Cardiomyopathy of Pregnancy

SIR,—In their report of a primipara with fatal brain damage associated with cardiomyopathy of pregnancy (2 November, p. 285) Dr. I. M. Ledingham and his colleagues stated that exhaustive investigations in this patient had been unsuccessful in identifying a specific aetiological factor. However, it seems relevant to mention that we had obtained serological evidence of active infection with herpes simplex virus during this patient's illness.

Her neurological symptoms started on 16 January 1967. Serial specimens or sera collected during her illness and tested in parallel showed a rising complement-fixing titre for herpes simplex—namely, 1 in 16 (16 January), 1 in 64 (28 January), 1 in 1,024 (18 February), and 1 in 512 (15 March). The complement-fixing titre for varicella-zoster showed only a twofold rise from 1 in 8 to 1 in 16 during the whole period. We were also able to obtain a specimen of serum which had been collected for routine antenatal tests on 26 December 1966, before her illness began. This showed the same titre for herpes simplex as that collected on 16 January at the beginning of her illness. This

suggests that the herpes infection during her illness was not primary but a reactivation of a latent virus.

The precise association of this herpes simplex infection to her cardiomyopathy is uncertain. As herpes simplex is an "opportunistic" virus it may well have been a secondary manifestation. Moreover, herpes simplex infection has not been found in association with myocardial involvement, either in our own laboratory experience or to my knowledge by other workers. However, it seems possible that primary infection or reactivation of a latent herpes simplex infection during pregnancy may cause cardiomyopathy. This possibility seems worth investigating by serological tests in other cases of cardiomyopathy of pregnancy.—

CONSTANCE A. C. ROSS.

Regional Virus Laboratory,
Ruchill Hospital,
Glasgow N.W.

Infusion of Liver Tumours

SIR,—We read with interest the leading article on infusion of liver tumours (10 August, p. 329), as well as the article by Mr. J. L. Provan and others (10 August, p. 346). We agree that "some of these questions may be answered more readily in countries where malignant hepatoma is commoner. . . ." We have now treated 20 patients in this way.

Controlled therapeutic trials on the treatment of primary hepatoma in the Bantu have been in progress at the City Deep Mine Hospital in Johannesburg for several years.¹⁻³ One hundred and ninety patients with hepatoma have been studied. Systemic cancer chemotherapy using many well-tried preparations, radiotherapy, and radiotherapy plus chemotherapy have been disappointing. There has, however, been extended survival time in all treatment groups as compared with vitamin C-treated randomized controls. One of us (G.F.) treated an additional 103 patients with primary liver cancer with various cytotoxic drugs with equally poor results. Our technique of liver infusion has been based on that of Sullivan and Watkins.^{4,5} We use the Watkins Chronofusor by choice as the most accurate infusor apparatus available. Heath Robinson methods such as suspending a Vacolite by a hook from the ceiling, besides being dangerous (air embolism), are a trial to patients, doctors, and nursing staff. Even the Bowman pump is not without danger, and the patient must be confined to bed. The Fenwal apparatus is better, since all air can be expelled from the plastic bag when filling it with infusate, and it is light and portable although rather bulky. But the patient certainly cannot go to work wearing apparatus such as this.

Since surgery has its limitations, hepatic artery infusion is the logical route to try next. An angiogram is a prerequisite. Even with the aid of an angiogram and even when the hepatic artery is catheterized selectively, there is a lack of specific regional infusion in the liver. In Bantu patients with malignant hepatoma the liver is hard and friable, and its size obstructs surgical approach to the artery through the lesser omentum. Catheterizing the right gastro-epiploic artery is, however, a simple matter. The right gastric artery is ligated to prevent infusate reaching the stomach. Disulphine blue dye injected through the catheter shows where the infusate will go. Brachial artery and femoral artery catheterization do not provide the essential information. A Teflon catheter can be left in for many months. Many repeat courses of

treatment can be given, alternating with sterile water, or the end of the catheter may be occluded and reopened at will, simply by forcible injection of saline in a syringe.

Regarding the question of choice of drug, the answer will be forthcoming from careful documentation of all data. At the present time methotrexate given intra-arterially gives the best response in our series. Dosage schedules and duration of treatment must be compared and correlated against results in terms of useful life. From such data the best schedule for adequate trial of therapy for malignant hepatoma can be worked out. The time has passed when a single case report or

reports on groups of two or three patients are adequate.—We are, etc.,

GEOFFREY FALKSON.
E. W. GEDDES.

Department of Cancer
Chemotherapy,
University of Pretoria,
South Africa.

REFERENCES

- South African Primary Liver Cancer Research Group—initial report, *S. Afr. med. J.*, 1967, 41, 399.
- Coetzee, A. M., A.R. *Brit. Emp. Cancer Campgn.*, 1966, 44, 356.
- Coetzee, A. M., A.R. *Brit. Emp. Cancer Campgn.*, 1967, 45, 380.
- Sullivan, R. D., 1967, personal communication.
- Watkins, E., 1968, personal communication.

Streptomycin and C.S.F. Sugar

SIR,—In the bacterial meningitides the cerebrospinal fluid cell count and sugar concentration are the most useful indices of the effectiveness of therapy. However, Brain¹ observed that measurements of C.S.F. sugar were unreliable in tuberculous meningitis treated with intrathecal streptomycin. This observation was made before the glucose oxidase method for measuring glucose became available. We have investigated therefore the effect of streptomycin on the level of C.S.F. sugar estimated by glucose oxidase and ferricyanide methods. Preliminary experiments showed that in 18 specimens of C.S.F. the addition of streptomycin to a con-

centration of 100 mg./100 ml. caused an apparent rise of 22% of C.S.F. sugar measured by an AutoAnalyzer modification of the Hoffman² ferricyanide method, while in 10 specimens assayed by a glucose oxidase method³ the addition of streptomycin did not alter the C.S.F. sugar concentration.

	AutoAnalyzer Ferricyanide Method	Manual Ferricyanide Method	Glucose Oxidase Method
C.S.F. Mean sugar concentration, mg./100 ml.	71.5 ± 8.1*	83.8 ± 10.2†	68.7 ± 10.6†
C.S.F. and Strept. Mean sugar concentration, mg./100 ml.	82.3 ± 12.2	79.9 ± 13.0	62.6 ± 10.8
C.S.F. and Strept. Corrected for dilution.	90.7 ± 13.3*	87.9 ± 13.8†	68.9 ± 11.8†
Mean sugar concentration, mg./100 ml.	+26.6%	+4.9%	+0.3%
Change due to streptomycin			

* P < 0.0005. † Not significant.

centration of 100 mg./100 ml. caused an apparent rise of 22% of C.S.F. sugar measured by an AutoAnalyzer modification of the Hoffman² ferricyanide method, while in 10 specimens assayed by a glucose oxidase method³ the addition of streptomycin did not alter the C.S.F. sugar concentration.

Samples of C.S.F. from 15 patients were collected into fluoride-oxalate tubes in the course of routine investigations for a variety of neurological diseases. No patient had a meningitic illness. Two aliquots of 2 ml. were taken from each specimen, and to one was added 2 mg. of streptomycin sulphate in 0.2 ml. of solution. The sugar concentration in each aliquot was analysed by a glucose oxidase method,⁴ by the AutoAnalyzer modification of Hoffman's method,² and by a manual ferricyanide method.⁵ The mean sugar concentration (± standard deviation) measured by each technique is shown in the Table.

After allowing for dilution, the mean C.S.F. sugar concentration by the AutoAnalyzer method was 26% higher in the streptomycin-containing aliquots than in the controls, and this difference was statistically highly significant. Though streptomycin produced a small rise of 4.9% in the mean sugar concentration measured by a manual ferricyanide method, neither with this nor with a glucose oxidase method was the change statistically significant.

Streptomycin is a compound of streptidine and a disaccharide, streptobiosamine.⁶ How-

ever, the chemistry of oxidation of streptomycin by ferricyanide is more complex than the simple oxidation of this disaccharide, for the exact experimental conditions alter the result. With the AutoAnalyzer method one mole of glucose is approximately equivalent to three moles of streptomycin. The quantity of streptomycin added to the aliquots was chosen to produce a concentration in the C.S.F. of about twice the maximum expected after an intrathecal injection of 50 mg. of streptomycin. It is concluded that the C.S.F. sugar concentration can be used as an index of the activity of the disease during the intrathecal treatment of tuber-

culous meningitis with streptomycin provided that a glucose oxidase assay method is used.—We are, etc.,

W. G. BRADLEY.

D. R. AYYAR.

A. H. DE C. FREED.

I. L. WOOLF.

Regional Neurological Centre,
Newcastle General Hospital,
Newcastle upon Tyne.

A. J. CASSELLS-SMITH.

Department of Clinical
Biochemistry,
Newcastle General Hospital,
Newcastle upon Tyne.

R. H. WILKINSON.

Nuffield Department of
Clinical Biochemistry,
Radcliffe Infirmary,
Oxford.

REFERENCES

- Brain, Lord, *Diseases of the Nervous System*, 1962, 6th ed., p. 334. London.
- Hoffman, W. S., *J. biol. Chem.*, 1937, 120, 51.
- Discombe, G., *J. clin. Path.*, 1963, 16, 170.
- Raabo, E., and Terkildsen, T. C., *Scand. J. clin. Lab. Invest.*, 1960, 12, 402.
- Haslewood, G. A. D., and Strookman, T. A., *Biochem. J.*, 1939, 33, 920.
- Goodman, L. S., and Gilman, A., *Pharmacological Basis of Therapeutics*, 1955, 2nd ed. p. 1360. New York.