were cured, and in fact the results have proved so disappointing that it has been reluctantly decided to abandon the trial.

Further work might show whether aminotroazole is effective in eliminating trichomonads in the female urinary tract and in the male, but the results so far suggest that it is not suitable alone for vaginal infections. There may be a place for it in conjunction with an effective trichomonicide, used locally in the vagina, especially in chronic cases, but its use without such treatment does not give sufficiently good results to make it a practical method.

We acknowledge our indebtedness to Ortho Pharmaceutical Limited for their generous support of this investigation. This has included help with the technical side and supplies of tritheon, aci-jel, and materials for the culture medium. This help has been given without prejudice to any results that might be obtained. We also thank Mr. D. Innes Williams and the staff of St. Paul's Hospital. Miss Landau, Miss Mocatta, and Miss Hurter kindly allowed us to treat patients under their care.

REFERENCES

- Cuckler, A. C., Kupferberg, A. B., and Millman, N. (1955). *Antibiot. and Chemother.*, 5, 540.
Kean, B. H., and Day, E. (1954). *Amer. J. Obstet. Gynec.*, 68, 1510.
Kupferberg, A. B., Johnson, G., and Sprince, H. (1948). *Proc. Soc. exp. Biol. (N.Y.)*, 67, 304.
Perl, G., Guttmacher, A. F., and Raggazoni, H. P. (1956). *Obstet. Gynec.*, 7, 128.
Plaut, A. A., Gray, M. J., Neslen, E. D., and Dalali, S. J. (1956). *Amer. J. Obstet. Gynec.*, 71, 116.

SENSITIVITY TO FOLIC ACID

BY

I. CHANARIN, M.D., D.C.P.

Registrar

J. C. B. FENTON, M.B., D.C.P.

Registrar

AND

D. L. MOLLIN,* M.B.

Lecturer

Department of Pathology, Postgraduate Medical School of London, W.12

Folic acid (pteroylglutamic acid) has been extensively used since 1945 not only for the treatment of certain megaloblastic anaemias but also as an ingredient of numerous vitamin preparations. In spite of this wide use the only report of sensitivity to this drug is that of Mitchell, Vilter, and Vilter (1949). Their patient, a woman aged 35, reacted on two occasions to folic acid. On the first occasion she developed a pruritic rash which disappeared on withdrawal of the drug; on the second occasion an anaphylactoid reaction followed an intravenous dose. Subsequent skin tests confirmed that she was sensitive to folic acid. She was known also to be sensitive to phenobarbitone, various foods, wool, soaps, and face-powder.

The purpose of this paper is to report an example of sensitivity to folic acid arising in a previously healthy subject. The results of intradermal tests with folic acid and closely related compounds are also described.

Case Report

A healthy male doctor aged 35 was given, for experimental purposes, 3 mg. of folic acid as a solution of the sodium salt by mouth without untoward reaction. The material was absorbed normally, for the serum folic-acid concentrations, measured with *Streptococcus faecalis* R assay (Jukes, 1955), rose from 0 to 40 μ g. per ml. in the first four hours after the dose.

*In receipt of a grant from the Medical Research Council.

Six weeks later the same subject was given four 5-mg. tablets of folic acid by mouth. Forty minutes after taking the tablets he complained of general malaise, aching pain in the lower thoracic region, and respiratory difficulty with restricted inspiration. This was followed 20 minutes later by itching of the palms of the hands and soles of the feet. Pruritus then became generalized; it was worse at the flexures, and was associated with a generalized erythematous rash, most pronounced on the thighs and forearms. The reactions disappeared within three hours of taking the folic acid.

Investigations.—Intradermal tests were carried out by injecting 0.1 ml. of the test solutions into the skin on the front of the forearm. A test solution of folic acid was included in each set of tests together with a distilled-water control. Positive reactions with weal, red flare, and itching were obvious in 10 to 15 minutes after the injection; they reached a peak in about 20 minutes and disappeared in 45 to 60 minutes. The substances tested, the concentrations of test solutions, and the results are given in the Table.

Two different samples of commercially available folic acid (pteroylglutamic acid) gave positive reactions after intradermal injection in concentrations of 5 μ g. per ml. or more, as did similar concentrations of a crystalline preparation of folic acid of high (97%) purity kindly supplied by Dr. William L. Williams, of Lederle Laboratories Inc. Similar reactions followed the intradermal injection of the same concentrations of aminopterin (4-aminopteroylglutamic acid). On the other hand, no reactions followed the intradermal injection of the following solutions: two preparations of folinic acid ("leucovorin," N⁵-formyltetrahydropteroylglutamic acid), N¹⁰-formyltetrahydropteroylglutamic acid; anhydroleucovorin; a folic acid conjugate "teropterin"; and amethopterin (4-amino-N¹⁰-methylpteroylglutamic acid).

Free antibody was tested for by a precipitation method using a constant antibody optimal-proportions technique (Dean and Webb, 1926) and also by adsorbing folic acid to tanned red cells and testing the ability of the patient's serum to agglutinate these cells (Boyden, 1951). No antibodies were demonstrated by either method.

Six months after the development of sensitivity the blood count was: red cells, 5,300,000 per c.mm.; haemoglobin, 15.4 g. per 100 ml.; packed cell volume, 46%; white cells, 8,000 per c.mm. There were no abnormalities in the stained film.

Discussion

The results of skin tests in our patient show that while he was sensitive to folic acid (pteroylglutamic acid) and its analogue aminopterin (4-aminopteroylglutamic acid), he did not react to folinic acid, anhydroleucovorin, N¹⁰-formyltetrahydropteroylglutamic acid, and amethopterin, which are related compounds with substituents in the 5 and 10 positions (see Table).

None of the compounds we used was absolutely pure; the purest folic acid obtainable contained about 3% impurities, and the aminopterin and related compounds contained traces of folic acid as well as other chemical contaminants.

Results of Intradermal Tests with Folic Acid and Related Compounds

Substance	Concentration (μ g. per ml.)	Result
Commercial folic acid (a) ..	{ < 5 5 to 100	— +
" " " (b) ..	{ < 5 5 to 100	— +
Purified " " " ..	{ < 5 5 to 100	— +
Aminopterin	{ < 5 5 to 100	— +
Folinic acid (a) (leucovorin) ..	250	—
Folinic acid (b)	250	—
Anhydroleucovorin	Saturated solution	—
N ¹⁰ -formyltetrahydropteroylglutamic acid	250	—
Teropterin	250	—
Amethopterin	250	—

It was therefore of some importance that a positive reaction was obtained with both folic acid and aminopterin at equal concentrations of 5 µg. per ml. and that at greater dilutions the reactions became doubtful with both substances. The patient reacted to the aminopterin *per se* and not to traces of folic acid which might have been present as a contaminant.

The formation of specific antibodies against folic acid by the subject studied indicates that this substance functioned in him either as an antigen or, more probably, as a haptene or antigenic determinant in a larger complex. An essential criterion of antigenicity is that the compound in question is "foreign" to the subject. These observations therefore suggest that pteroylglutamic acid was not normally present in the tissues of our "patient." As he remained perfectly well, however, and was not anaemic the requirement of his tissue cells for "folic acid" on this hypothesis must have been supplied by substances other than pteroylglutamic acid.

It has been recognized for some time that pteroylglutamic acid is itself unlikely to play a part in tissue metabolism. Recent work suggests that N¹⁰-formyltetrahydropteroylglutamic acid or a closely related compound is the physiologically active member of this group of substances (Greenberg, 1954). N¹⁰-formyltetrahydropteroylglutamic acid is itself highly unstable and probably exists in the liver in a bound stable form (Greenberg, 1954). Under aerobic conditions it is rapidly oxidized to N¹⁰-formylpteroylglutamic acid, and this in turn, under alkaline conditions, is converted to pteroylglutamic acid. Under anaerobic conditions, on the other hand, N¹⁰-formyltetrahydropteroylglutamic acid is converted to N⁵-formyltetrahydropteroylglutamic acid (folinic acid).

The isolation of folic or folinic acid from foodstuffs does not necessarily imply that this was the form in which this substance was originally present. It is possible that the absence of sensitivity reactions in our subject after meals is due to the fact that pteroylglutamic acid is not absorbed from food as such, and not that too little is absorbed to produce these reactions.

Summary

A subject is described who was found to have become sensitive to folic acid six weeks after taking an oral dose.

Intradermal tests showed that he was sensitive to folic acid and aminopterin, but not to folinic acid (leucovorin), anhydroleucovorin, N¹⁰-formyltetrahydropteroylglutamic acid, a folic acid conjugate (teropterin), or to amethopterin.

We thank Professor J. V. Dacie for advice and encouragement; and Dr. William L. Williams, of Lederle Laboratories Inc., for supplying the pure specimen of pteroylglutamic acid used in the study and also for the anhydroleucovorin, folinic acid (leucovorin), and teropterin.

REFERENCES

- Boyden, S. V. (1951). *J. exp. Med.*, 93, 107.
Dean, H. R., and Webb, R. A. (1926). *J. Path. Bact.*, 29, 473.
Greenberg, G. R. (1954). *Fed. Proc.*, 13, 745.
Jukes, T. H. (1955). In D. Glick's *Methods of Biochemical Analysis*, 2, 121. Interscience, New York.
Mitchell, D. C., Vilter, R. W., and Vilter, C. F. (1949). *Ann. Intern. Med.*, 31, 1102.

A Society of Indexers has been formed in London, its principal objects being to improve the standard of indexing and secure some uniformity in its technique, to set up a panel of indexers, and to publish papers and notes on the subject. It is believed to be the first society of its kind in the world. The chairman, Mr. A. R. HEWITT (librarian of the Institute of Commonwealth Studies), is son of a former librarian of the Royal Society of Medicine. Among those elected to the council of the society was Miss D. F. ATKINS, librarian of the Postgraduate Medical School of London. Further information may be obtained from the hon. secretary, Mr. G. NORMAN KNIGHT, 4, Fitzroy Street, London, W.1.

A CASE OF CONJOINED TWINS

BY

T. WILSON RODDIE, M.B., B.A.O., M.R.C.O.G.

Senior Lecturer in Obstetrics and Gynaecology, University of Malaya, Singapore

Conjoined twins are rare and may at times offer serious difficulties to delivery. The following case seems of sufficient interest to merit publication, as the monsters were delivered intact vaginally. Frequently some form of mutilating operation is necessary before vaginal delivery can be effected, or a caesarean section may have to be done.

Case Report

A Cantonese woman aged 30 was admitted in labour to the Kangar Kerbau Hospital in the thirty-second week of her eighth pregnancy. All seven previous confinements had been normal and had resulted in live births.

On examination she was anaemic, her face and hands were puffy, and there was clinical oedema of her feet and ankles. The abdomen was the size of a full-term pregnancy and rather tense.

There seemed to be definite hydramnios and the uterus was contracting. The possibility of a multiple pregnancy was considered, though no foetal heart sounds could be heard with certainty. Her blood pressure was 160/100 mm. Hg. The urine contained a trace of albumin. Vaginal examination revealed a bulging bag of membranes, a cervix practically fully dilated, and something other than a head presenting—probably

Photograph of the omphalopagus.

feet. A straight x-ray film of her abdomen showed twin foetuses both presenting by the breech. However, the fact that the two heads were on the same level and facing each other suggested the possibility of some form of foetal union.

By this time the patient was beginning to bear down and the membranes ruptured spontaneously. A large quantity of dark-coloured liquor amnii drained away and two feet presented at the vulva. It was decided to attempt delivery and she was anaesthetized. Vaginal examination now confirmed that the twins were joined together, and there did not seem to be any second sac, as the other pair of lower limbs and feet were felt at the cervix. These were brought down easily, and with traction on the four limbs the bodies descended parallel to each other through the dilated cervix and vagina. The legs and trunks were now lifted well forward over the symphysis pubis, allowing the posterior head to engage in the pelvis and be born with the anterior head following quickly afterwards. Both foetuses were fresh, and the one whose head was delivered last made feeble attempts to breathe. A single placenta, with velamentous insertion of two cords at opposite sides, was expressed a few minutes after delivery of the monster. There was no excessive blood loss, and episiotomy performed during the delivery was repaired with catgut. The puerperium was uneventful.