J. Physiol. (1954) 123, 603–617

THE MODE OF ACTION OF FOLIC ACID ANTAGONISTS ON CELLS

By W. JACOBSON*

From the Strangeways Research Laboratory, Cambridge

(*Received* 15 *October* 1953)

The usefulness of folic acid antagonists in the treatment of acute leukaemia and allied conditions was established by Farber, Diamond, Mercer, Sylvester & Wolff (1948) and Farber (1949); many papers have followed their original observation; the work has recently been summarized by Farber (1952).

The fact that in some cases of acute leukaemia the pathologically immature white cells are attacked by this group of antagonists indicates that either folic acid or one of its derivatives may be essential for the life of these cells. The results so far reported have shown that folic acid is required by many tissues for their maintenance and growth. The most recent review is by Bessey, Lowe & Salomon (1953).

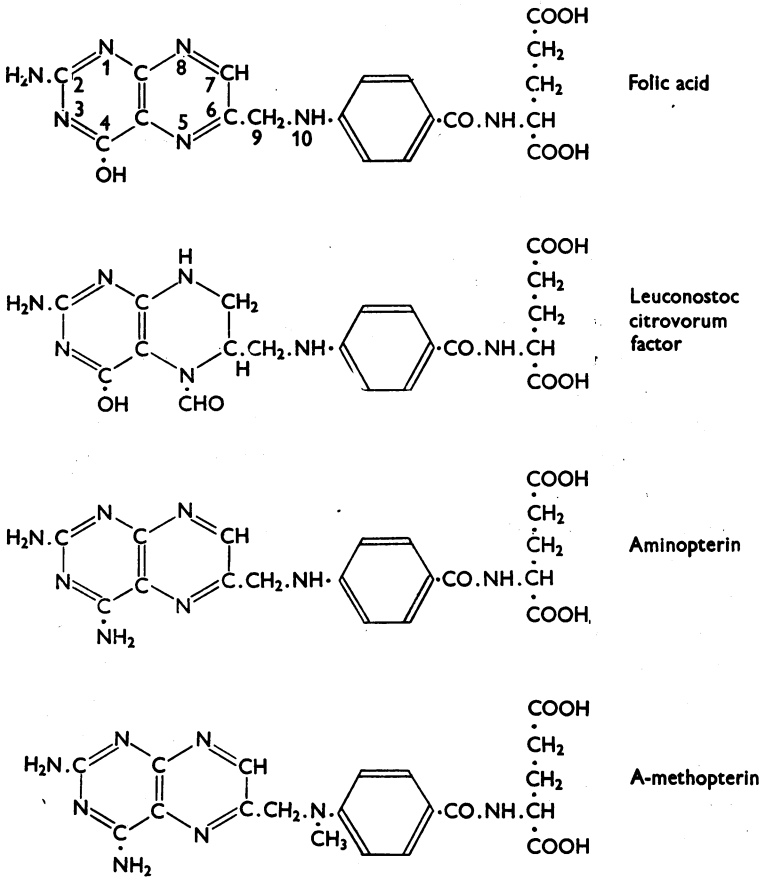
Very little is known, however, about the cellular processes which directly depend on the normal functioning of folic acid and its derivatives and which are blocked by the action of folic acid antagonists, though many indirect metabolic effects have been noted.

In this and a subsequent paper, experiments will be reported to show that one of the principal modes of action of folic acid antagonists is to interfere with the function of the *Leuconostoc citrovorum* factor (LCF) in the growth of both normal and pathological cells. Evidence will be presented to show that LCF, a member of the folinic acid group of compounds, plays an essential part during mitotic division and that, without this factor, cells which have entered mitosis are unable to advance beyond the metaphase stage. Short accounts of part of this work have been published elsewhere (Jacobson, 1950, 1951, 1952, 1953; Jacobson & Webb, 1950, 1952*a*).

There is an extensive literature on folic acid, pterines and folic acid antagonists. That concerning folic acid itself up to the beginning of 1947 has been summarized by Jukes & Stockstad (1948) and by Berry & Spies (1946); much information has also been published in the symposium on folic acid of the New York Academy of Science (SubbaRow, 1947), where the synthesis of folic acid (pteroylglutamic acid) by SubbaRow and his team is described. The

* Sir Halley Stewart Research Fellow.

chemistry of the pterines has been reviewed up to 1947 by Marshall Gates (1947), and more recently by Albert (1951, 1952). The work on folic acid antagonists has been comprehensively reviewed by Petering (1952) and in symposia edited by Woolley (1950) and by Farber (1952).



Text-fig. 1. In all formulae the pteridine ring is on the left, the *p*-amino-benzoyl-glutamic acid moiety towards the right.

Among the first antagonists prepared in 1947 and 1948 were aminopterin and A-methopterin (Seeger, Cosulich, Smith & Hultquist, 1949). The most effective antagonists used so far are those in which the OH-group in the 4-position of the pteridine ring is replaced by an amino-group. Aminopterin, the most powerful antagonist, is 4 : amino-pteroyl glutamic acid and A-methopterin, the activity of which is about one-fifth to one-tenth of that of aminopterin, is 4 : amino, 10 : methyl-pteroyl glutamic acid. The experiments described below were made with these two substances (see Text-fig. 1).

MATERIAL AND METHOD

Bone marrow. Smears and sections of bone marrow from transplantable acute mouse leukaemia before and after treatment with folic acid antagonists were studied. The smears were stained with May-Grunwald and Giemsa stain. In the ordinary air-dried smears it was noted that the chromosomes in all stages of division were swollen and stained somewhat diffusely. When, however, the smears were placed in absolute methanol just before they were quite dry and stained in the ordinary way, the chromosomes were well preserved and stained differentially, as will be described later. It is essential that the smears should not be completely dried as apparently the chromosomes swell during the last stage of desiccation. Sections also were stained by the same method after fixation in Zenker-formol or methanol.

Intestine. As folic acid antagonists may cause diarrhoea and lesions in the intestinal mucous membrane in man, this tissue was studied in the mouse; formol-saline or absolute methanol was used as a fixative, the latter on small pieces to fix the cytoplasmic ribo-nucleoproteins.

Tissue cultures. Since it was found that one of the main effects of the antagonists was on cell division, cultures of various tissues from chick embryos were grown *in vitro*, as very abundant mitoses appear in such material after 24–48 hr incubation. Over 450 cultures were used, mainly derived from the frontal bone, heart or sclerotic of 12-day-old chick embryos. A few cultures of skin, liver, spleen and spinal cord of chick embryos were also made, and of adult mouse bone marrow, leukaemic cells of an acute transplantable mouse leukaemia of an FAK_1 strain and of a transplantable mouse sarcoma. All cultures were grown in a hanging drop of a mixture of equal parts fowl plasma and chick embryo extract (of 12-day-old embryos). Approximately 0·01 ml. both of plasma and embryo extract provided the medium which was placed on $\frac{7}{8}$ in. square cover-slips. The cultures were incubated at 37° C. As by far the most frequent mitoses occurred in the 24- to 48-hr-old cultures derived from frontal bone, heart and the sclerotic, and as the same general effects of folic acid antagonists were observed on dividing cells of all the tissues examined, the detailed analysis of the effects was made on osteoblasts and fibroblasts.

Only in a few experiments was the test substance added to the medium from the beginning of the incubation. Generally a small drop, 0·01–0·02 ml., of the test substance was placed on the area of the explant in the plasma clot of 24 or preferably 48 hr cultures, which contained, usually, a very high number of cells in division. The following precautions had to be taken, especially in the short-term experiments lasting from 15 min to 2 hr.

(1) The test substance had to be placed accurately on the area of the explant.

(2) Undue cooling of the cultures had to be prevented as chilling to room temperature delayed cells entering into the prophase and prolonged the duration of metaphase. The slides carrying the cultures were held by their edges only and were not placed on the bench. The seal of soft wax round the cover-slip was broken on three sides, the cover-slip prised up, the droplet of test fluid deposited on the tissue and the cover-slip placed again in its correct position. The wax around the edges of the cover-slip was pressed down and the slide was put back into the incubator. If this work is being done in front of the incubator door, a slide carrying a culture is exposed to room temperature for less than 30 sec, and this has no or very little effect on the cells. Sometimes, particularly in large-scale experiments, prophases were slightly fewer in control cultures, treated with saline or serum only without any inhibitor, when compared with the untreated controls which had been fixed immediately on being taken out of the incubator.

Test solutions were dissolved either in Tyrode or in a mixture of equal parts of serum and Tyrode. The concentrations of the test substances given throughout this work refer to the concentrations in the test solution. As the drop of the test solution placed on the cultures was of the same order of volume as the medium in which the cells were growing, it is probable that the cells were actually exposed to a concentration of the test substance lower than that in the drop placed on the explant.

The cultures were fixed in absolute methanol for at least 5 min. (They can be kept in this fixative for 1 or 2 days if required.) The cultures were stained with undiluted May-Grunwald

solution for 10 min, followed by Giemsa diluted 1 : 15 with glass-distilled water for 20 min and were then washed for 1–5 sec in glass-distilled water, dehydrated rapidly in two changes of acetone followed by acetone-xylol in equal parts, and cleared in xylol. This technique, which has been described in detail elsewhere (Jacobson & Webb, 1952*b*), gave an excellent differential stain for the nucleoproteins in dividing cells, ribonucleoproteins appearing dark blue and deoxyribonucleoproteins being stained red-purple.

RESULTS

The effect of folic acid antagonists on bone marrow from acute human and mouse leukaemia

Mitoses are infrequent in smears of human bone marrow (usually less than 1%), so that 16,000 nucleated cells had to be counted in order to classify the dividing cells among them into the four stages: pro-, meta-, ana-, and telophase. During prophase the chromosomal threads are still surrounded by the nuclear membrane and the nucleolus is still visible, but in metaphase (Pl. 1, fig. 2), the chromosomes are gathered in a central region in the cytoplasm, and the nuclear membrane and the nucleolus have disappeared. During anaphase, the two sets of chromosomes for the two daughter cells move apart but the cytoplasm is not yet divided; in telophase the cytoplasm for the two daughter cells is either nearly or completely separated, and the two resting nuclei are in the process of reconstruction.

The interference by folic acid antagonists with the normal completion of cell division was shown in the bone marrow in cases of acute human and mouse leukaemia. Thus, in a case of acute human leukaemia, the percentage distribution of the four mitotic phases before and 24 hr after the injection of 5 mg A-methopterin indicated that the leukaemic cells entered mitoses at an approximately normal rate but were arrested in metaphase, only a few proceeding to ana- and telophase. The results are shown in Text-fig. 2 (16,000 cells counted for each bone marrow).

Twelve similar observations were recorded.

In an acute mouse leukaemia a relatively much higher dose of folic acid antagonist could be employed (1 mg aminopterin) and the bone marrow was examined 2 hr after its subcutaneous injection (Pl. 1, fig. 1). Thus a greater effect could be produced. The percentage distribution of mitotic phases in the bone marrow of leukaemic mice before and 2 hr after the subcutaneous injection of 1 mg aminopterin is shown in Text-fig. 3. The two graphs are based on 16,000 leukaemic cells from two untreated animals and on the same number of cells from two treated mice.

From these observations it was concluded that folic acid antagonists interfere with the processes which lead from the metaphase to the anaphase stage of division in the leukaemic cells. The question arose whether this inhibition applied generally to dividing cells in normal tissues, and experiments were made to determine this point.

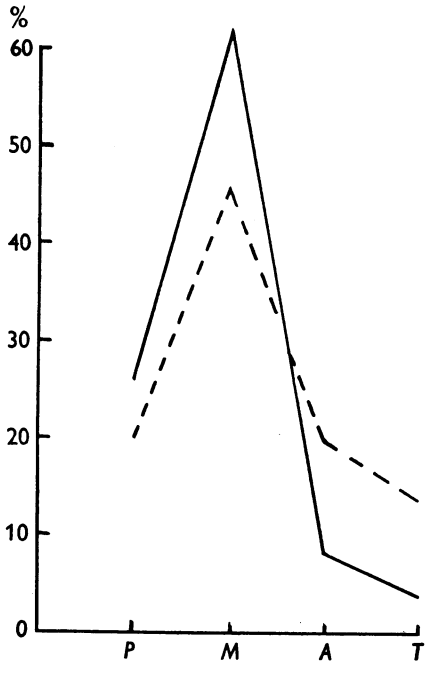


Fig. 2.

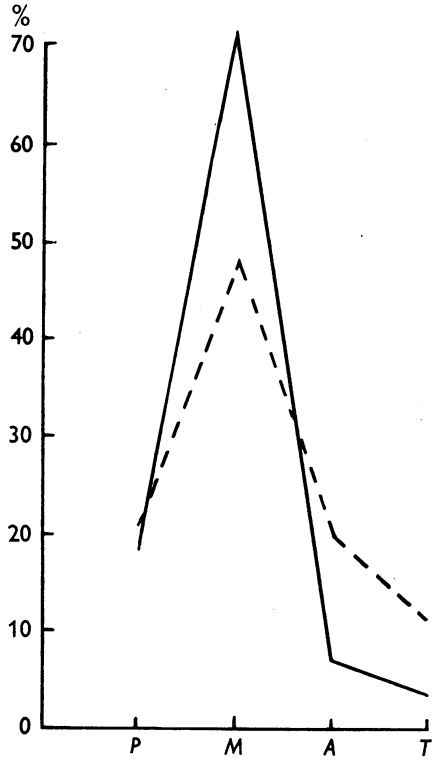


Fig. 3.

Text-fig. 2. Percentage distribution of mitotic phases in a bone marrow of a case of acute leukaemia before (– – –) and 24 hr after (——) 5 mg A-methopterin. *P*, prophase; *M*, metaphase; *A*, anaphase; *T*, telophase.

Text-fig. 3. Percentage distribution of mitotic phases in the bone marrow of leukaemic mice before (– – –) and 2 hr after (——) the injection of 1 mg aminopterin. (Abbreviations as in Text-fig. 2.)

The effect of folic acid antagonists on the intestinal epithelium of the mouse

The intestinal epithelium with its numerous mitoses in the glands of Lieberkühn, was very favourable material for studying the effects of folic acid antagonists on cell division. There were already indications that these agents affected the intestine, as occasionally during treatment, diarrhoea and specific lesions had been observed. When aminopterin in a dosage of 0·2 mg was injected subcutaneously into mice it was found that the mitoses in the glands of the small intestine were severely affected within 2 hr. Pathological forms such as tightly clumped chromosomes in metaphase were common (Pl. 1, figs. 4, 5). A normal metaphase is shown on Pl. 1, fig. 3. After 3 days the effect of the failure to replace epithelial cells showed itself in the glands where there were fewer cells per unit area of the epithelial lining. In some glands in

which the lumina were greatly distended the remaining cells were thinned out to an extraordinary degree, giving the impression of a very thin squamous epithelium instead of the regular columnar lining. Other glands with less distended lumina were lined by cells with abnormally large nuclei and an increased amount of cytoplasm; they were still connected with the surface epithelium by a thin strand of epithelial cells, but it was impossible to find such connexions in some of the cystically dilated glands (Pl. 1, fig. 7), where it may be assumed that not enough cells were available to provide continuity with the surface.

Aminopterin also produced a striking effect on the epithelium of the villi; the latter were much contracted and were covered by very large, vacuolated cubical cells instead of the tall prismatic type normally present. About ten to twenty of these large cells were sufficient to cover a villus in longitudinal section (Pl. 1, fig. 7), while normally about 100 or more cells were required (Pl. 1, fig. 6). Such changes had already appeared 3 days after a single dose of aminopterin, and some of the enlarged epithelial cells were degenerating and being shed. During the next few days patches of this epithelium often broke down completely so that the mucous membrane became denuded of its protective layer. This in turn resulted in bacterial invasion and led to diarrhoea and septicaemia from which death resulted. This was found to be the immediate cause of the animals' death.

These observations were particularly interesting, because in patients treated with folic acid antagonists, diarrhoea may occur and post-mortem findings in such cases reveal very similar changes in the glandular epithelium of the intestinal mucous membrane.

Observations on the effect of folic acid antagonists on cells grown in vitro

The effect of short exposures

Fibroblasts from the heart or sclerotic and osteoblasts from the frontal bones of 12-day chick embryos, when grown *in vitro* for 24–48 hr, were found to be the most suitable types of tissue culture for these experiments, owing to the ease with which they could be produced and the very large number of dividing cells which they contained. The results described below, however, apply to all the cell types examined, whether epithelial or mesenchymatous, normal or malignant.

As in the following experiments a small drop of the test solutions was placed on the explants it was necessary to determine (1) whether the dissolving medium alone would have an effect on the mitotic phases, and (2) whether the age of the cells growing *in vitro* altered in any way the distribution of these phases.

(1) Either Tyrode or a mixture of equal parts serum and Tyrode was used

as the dissolving medium for the test substances throughout this investigation. Table 1 shows that there is no significant difference in the distribution of mitotic phases of untreated cultures and those treated for 15 min with either Tyrode or a mixture of serum and Tyrode.

TABLE 1. The percentage of mitotic phases in tissue cultures untreated, exposed for 15 min to Tyrode, or Tyrode and serum equal parts

	Untreated	Tyrode	Tyrode + serum equal parts
Prophase	17%	15%	15%
Metaphase	38%	38%	37%
Anaphase	21%	23%	23%
Telophase	24%	24%	25%
No. of mitoses	621	584	314
No. of cultures	5	5	4

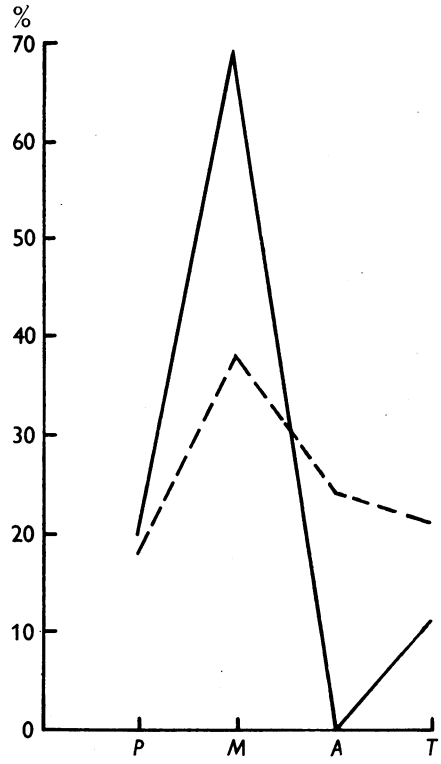
(2) Nine control cultures maintained through a second passage of 48 hr gave the following percentage distribution for the four mitotic phases: 18, 38, 24 and 20 among 3159 cells in division. It was therefore assumed that considerable deviations from these figures could not have been caused by the experimental procedure as such, and that the percentage distribution of mitotic phases remained substantially unaltered during the period of growth. These assumptions were fully borne out in the course of the investigation, as mitotic counts showed that in forty consecutive experiments the control cultures treated with the dissolving medium alone had consistently the same pattern of distribution of mitotic phases.

When a solution of 1 : 2000 aminopterin was applied to actively growing tissue cultures, within 15 min profound changes occurred in the distribution of the mitotic stages. There was a marked accumulation of cells in metaphase, and anaphases were either absent or extremely rare. The chromosomes in metaphase were arranged in a small, tight cluster (Pl. 1, fig. 8) instead of in the normal metaphase plate (Pl. 1, fig. 9). The percentage values in a typical experiment for the four stages of cell division in the control cultures (14 with 2363 mitoses) and in those treated with aminopterin 1 : 2000 for 15 min (10 cultures with 961 mitoses) are given in text-fig. 4.

In the course of the present and subsequent investigation this experiment has been repeated on thirty separate occasions as the standard of aminopterin inhibition, with consistent results. This observation indicated that the folic acid antagonist does not prevent resting cells from entering division, though the prophase stage may sometimes be prolonged; the cells pass into the metaphase stage but here the changes are arrested, and do not proceed to anaphase. As no cells were found in anaphase, however, it appears that those already in anaphase at the beginning of treatment were not prevented from entering telophase. An anaphase in a normal culture is shown on Pl. 1, fig. 10.

By the May-Grunwald and Giemsa method of staining it is possible to distin-

guish deoxyribonucleoprotein, which appears purple-red, from the ribonucleoprotein, which stains blue. The initial stage of cell division, the prophase, proceeded normally when it was exposed to aminopterin; in early prophase the chromosomes could be seen as fine purple-red threads. They gradually thickened, and small areas appeared on them which carried blue-stained ribonucleoprotein in addition to the deoxyribonucleoprotein. Towards the end of pro-



Text-fig. 4. Percentage distribution of mitotic phases among chick embryo osteoblasts, growing *in vitro*. Untreated (– – –) and 15 min after treatment (——) with aminopterin 1 : 2000. (Abbreviations as in Text-fig. 2.)

phase the chromosomes contained both types of nucleoprotein throughout their length, and thus appeared nearly black. The nuclear membrane and the nucleolus disappeared. Up to this point no deviation from the normal sequence (Jacobson & Webb, 1952*a*, *b*) could be found in the treated cells. The first abnormality became apparent in metaphase when the chromosomes were lying in the cytoplasm; they aggregated in a tight cluster and failed to split, so that no anaphase occurred. Those cells, however, which were found in telophase contained normal-looking daughter nuclei, which re-formed a nuclear membrane and nucleoli (Pl. 1, fig. 8); the chromosomes within the telophase

nuclei have lost most, but not yet all, of the ribonucleoprotein and therefore appeared purple-red over most of their length. The normal processes within the telophase nuclei do not seem to be affected by the antagonist, though sometimes it was noted that the cytoplasm failed to divide. In this way binucleated cells were occasionally formed.

If the solution of 1 : 2000 aminopterin was allowed to act on the cells for a longer period, after 2 hr a few cells might pass through anaphase and telophase, though there was still a great increase in number of cells in metaphase, nearly all of which showed the characteristic tight cluster of chromosomes.

When weaker solutions of aminopterin were used the same effect was observed, though a few cells were found in anaphase (Table 2).

TABLE 2. Effect of aminopterin 1 : 5000 and 1 : 10,000 on mitotic phases

		15 min after	
	Controls	1 : 5000	1 : 10,000
Prophase	16%	15%	15%
Metaphase	38%	70%	64%
Anaphase	22%	3%	3%
Telophase	24%	12%	18%
No. of mitoses	1205	976	544
No. of cultures	10	5	3

Aminopterin 1 : 20,000

	Controls	15 min after
Prophase	15%	16%
Metaphase	36%	62%
Anaphase	22%	6%
Telophase	27%	16%
No. of mitoses	857	1206
No. of cultures	4	4

Even in a dilution of 1 : 20,000 it is clear that the main effect of aminopterin was to arrest mitosis at metaphase, though not all cells concerned showed complete clumping of the chromosomes. Only few cells proceeded into anaphase and completed division.

Finally, when a solution of 1 : 40,000 aminopterin was applied to cultures of osteoblasts for 30 min and 1 hr respectively the results still indicated some characteristic inhibition, but not all metaphases presented the typical picture of clumped chromosomes and a number of anaphases proceeded normally (Table 3).

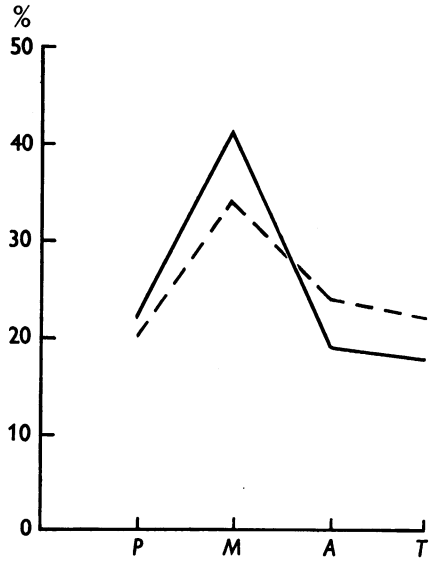
Solutions of aminopterin of 1 : 50,000 and less caused no significant inhibition in these experiments. It should be remembered that the volume of the test solution placed on the cultures was always kept between 0·01 and 0·02 ml., and the volume of the medium through which the inhibitor diffused was of the same range. Thus the concentration of aminopterin in actual contact with the cells would be considerably lower than in the test solution placed on the explant.

TABLE 3. Effect of aminopterin 1 : 40,000 on mitotic phases

	Controls	30 min after	1 hr after
Prophase	14%	17%	13%
Metaphase	40%	49%	51%
Anaphase	26%	14%	9%
Telophase	20%	20%	27%
No. of mitoses	1794	830	2255
No. of cultures	4	4	4

The effect of prolonged exposure to aminopterin 1 : 2000.

In view of the striking effect obtained in the short-term experiments on dividing cells *in vitro*, it was rather surprising to find that cultures grown in a medium containing aminopterin in a concentration 1 : 2000 showed a nearly normal distribution of the mitotic phases after 24 and even 72 hr, and their zone of outgrowth was almost indistinguishable from that of the controls (Text-fig. 5). This showed that the effect of even high concentrations of aminopterin gradually 'wears off'.



Text-fig. 5. Percentage distribution of mitotic phases among chick embryo osteoblasts growing *in vitro*. Untreated controls (– – –) and after exposure to aminopterin for 24 hr (——). The control graph was obtained from ten untreated cultures with 3159 mitoses; the aminopterin graph from six cultures with 810 mitoses. (Abbreviations as in text-fig. 2.)

Somewhat similar results were obtained with another folic acid antagonist, A-methopterin. This compound had been found by Farber (1952) to be as effective as aminopterin in the treatment of acute leukaemias, provided larger amounts were given; thus a daily dose of about 5 mg of A-methopterin was

required to produce the same effect in the patient as 1 mg aminopterin per day. Similarly, to cause the same degree of mitotic inhibition in tissue cultures, the dose of A-methopterin had to be five times as great as that of aminopterin. For instance, A-methopterin 1 : 400 arrested mitoses in metaphase within 1 hr; a few cells proceeded into anaphase and telophase. In one experiment 60% of all dividing cells were found in metaphase, whereas this figure for the control cultures was 38%, and the metaphase chromosomes showed the characteristic clumping. In short-term experiments, therefore, the action of A-methopterin was similar to that of the more powerful folic acid antagonist. Like aminopterin, A-methopterin (1 : 400) failed to retain its full inhibitory action after 24 hr contact with the tissue (Table 4). The impression was gained that the number of metaphases with clumped chromosomes was somewhat higher than in similar cultures treated with aminopterin.

TABLE 4. Effect of A-methopterin 1 : 400 for 24 hours on dividing cells

	Controls	Treated
Prophase	18%	20%
Metaphase	36%	46%
Anaphase	23%	17%
Telophase	23%	17%
No. of mitoses	2665	1674
No. of cultures	9	17

DISCUSSION

Folic acid antagonists are known to interfere with the development of chick embryos, the growth of young chicks and mammals, and with the normal function of bone marrow in birds and mammals. They prevent proliferation being induced by oestrogens or testosterone in organs of amphibia and birds and have some growth-restricting effects on certain tumours and leukaemic cells in mammals and man. They also inhibit the growth of some insect larvae and bacteria. This subject has been summarized by Petering (1952) and Woolley (1950). The metabolic processes directly blocked or restricted by the folic acid antagonists are unknown, but they must be widespread throughout living matter, from bacteria (Woods, 1953) to man.

The arrest of mitosis in onion root tips by aminopterin was reported in an abstract of a paper by Himes & Leuchtenberger (1949). Dustin (1950) noted that mitoses in normal bone marrow and intestinal epithelium are severely affected by aminopterin, and in this laboratory Hughes (1950) recorded by cinematography the clumping of metaphase chromosomes in living tissue cultures of embryonic chick fibroblasts. Hughes also observed cells arrested in metaphase which, without completing division, reconstructed a resting nucleus. This mechanism may be responsible for the formation of the large cells with swollen nuclei which were found in the intestinal epithelium.

Whereas these observations were interpreted as phenomena indicating specific metabolic requirements of dividing cells of a few selected types, it is now possible to say that the arrest of dividing cells in metaphase by folic acid antagonists ranges from human or mouse leukaemic cells to chick embryonic osteoblasts and may, therefore, be a general phenomenon among dividing cells.

The fact that after an initial arrest in metaphase some types of cells overcome the inhibition will be analysed in the following paper. It is not certain whether folic acid antagonists act primarily on the spindle fibres. Colchicine, which is considered to be a typical 'spindle inhibitor', causes either scattering or clumping of chromosomes, whereas folic acid antagonists cause clumping only. Grampa & Dustin (1952) observed spindle fibres in dividing cells of the intestinal epithelium which had been exposed first to aminopterin, and 3 days later to colchicine.

The results of the present investigation have shown that one important phase in cell division, viz. the processes which lead to the parting of the two chromosome halves, the chromatids, requires the function of a substance which is replaced by folic acid antagonists. Even in the presence of very high concentrations of aminopterin, cells enter the prophase of division and advance to metaphase; this means that the formation of chromosomes is not affected by folic acid antagonists. In this connexion it is of interest to mention that the chromosomes contain ribonucleoprotein towards the end of prophase, just before the nuclear membrane and the nucleolus disappear (Jacobson & Webb 1952*a*, *b*). This appearance of ribonucleoprotein on the deoxyribonucleoprotein-containing chromosomes is not affected by antagonists, and the metaphase chromosomes thus contain both types of nucleoprotein, even when exposed to high concentrations of aminopterin. On the other hand, those processes which keep the individual chromosomes apart, thus preventing them from clumping and leading eventually to the separation of the two chromatids, are affected by the folic acid antagonists, for in their presence the chromosomes clump and their chromatids do not separate.

In normal division, when the daughter chromosomes part from each other in anaphase, a relatively higher concentration of ribonucleoprotein is found in that area of the cytoplasm through which the daughter chromosomes have moved. This may be due to the shedding by the chromosomes of some of their ribonucleoprotein (Jacobson & Webb, 1952*a*, *b*). When cultures are treated with a low concentration of aminopterin the few cells found in anaphase behave in this respect like normal cells in mitosis. Thus the two phenomena of anaphase, the movement of the daughter chromosomes and the shedding by them of ribonucleoprotein, were not found separately.

The fact that low concentration of aminopterin arrested fewer cells in metaphase indicates that the inhibition is not an 'all or none' process, some

cells in an apparently homogeneous population being more susceptible than others.

At present it is not possible to determine which chemical processes are blocked by the folic acid antagonists, but it can be stated that these processes are essential for the parting of the chromosome halves, which takes place over a period of only a few minutes.

SUMMARY

1. The folic acid antagonist aminopterin, when used in sufficiently high doses, prevents dividing cells from completing mitosis; they are arrested in the metaphase stage and cannot proceed into anaphase. The metaphase chromosomes form a tight cluster.

2. Embryonic chick fibroblasts and osteoblasts growing *in vitro* provided a suitable test material with abundant cells in division.

3. The effect of aminopterin on dividing cells is fully developed within less than 15 min, when concentrations between 1 : 2000 and 1 : 20,000 are applied.

4. Cells are not prevented from entering into divison by folic acid antagonists.

5. Similar observations were made on smears from the bone marrow of cases of acute leukaemia in man and a transplantable acute mouse leukaemia.

6. The inhibitory effect of aminopterin wears off during 24 hr.

7. Similar observations were made with another folic acid antagonist, A-methopterin.

8. The intestinal mucous membrane with its high rate of dividing cells undergoes profound changes when exposed to aminopterin, and in the course of 5–6 days the villi become denuded of their epithelial cover. This was shown on the small intestine of the mouse.

The author wishes to express his gratitude to Dr H. B. Fell, F.R.S., Director of the Strangeways Research Laboratory, for her unfailing help and interest in the progress of this work, and to Dr S. Farber, Pathologist-in-Chief, The Children's Hospital, Boston, who during a stimulating period of well over a year gave full access to the extensive clinical and pathological material at his disposal. The help given by Miss Margaret M. Fell, now at The Children's Hospital, Boston, and Miss June Rawlinson, in making the tissue cultures and preparing the graphs for publication is acknowledged with gratitude. The author is indebted to Dr R. P. Parker, Director of Research, of the Calco Division of the American Cyanamid Company, for the generous supply of folic acid antagonists.

The financial support of the Rockefeller Foundation, the Fulbright Committee and the Medical Research Council is gratefully acknowledged.

REFERENCES

ALBERT, A. (1951). The pteridines. *Rev. pure appl. Chem.* **1**, 51–63.

ALBERT, A. (1952). The pteridines. *Quart. Rev. chem. Soc., Lond.*, **6**, 197–237.

BERRY, L. J. & SPIES, T. D. (1946). The present status of folic acid. *Blood*, **1**, 271–306.

BESSEY, O. A., LOWE, H. J. & SALOMON, L. L. (1953). Water-soluble vitamins. *Annu. Rev. Biochem.* **22**, 555–563.

DUSTIN, P. Jr. (1950). Lésions cellulaires provoquées par les acides 4-aminoptéroylglutamiques chez la souris. *Rev. Hémat.* **5**, 603–617.

FARBER, S. (1949). Some observations on the effect of folic acid antagonists on acute leukaemia and other forms of incurable cancer. *Blood*, **4**, 160–167.

FARBER, S. (1952). Proceedings of the second conference on folic acid antagonists in the treatment of leukaemia, ed. FARBER, S. *Blood*, **7**, 97–190.

FARBER, S., DIAMOND, L. K., MERCER, R. D., SYLVESTER, R. F. Jr. & WOLFF, J. A. (1948). Temporary remission in acute leukaemia in children produced by folic acid antagonist, 4-aminopteroylglutamic acid (aminopterin). *New Engl. J. Med.* **238**, 787–793.

GATES, M. (1947). The chemistry of the pteridines. *Chem. Rev.* **41**, 63–95.

GRAMPA, G. & DUSTIN, P. Jr. (1952). Analyse, par la colchicine, des effets radiomimétiques de l'acide 4-amino-ptéroylglutamique (aminoptérine). *Rev. belge Path.* **22**, 113–125.

HIMES, M. & LEUCHTENBERGER, C. (1949). The effect of some folic acid analogues on mitosis. *Rec. Genet. Soc. Amer.* p. 94.

HUGHES, A. F. W. (1950). The effect of inhibitory substances on cell division. *Quart. J. micr. Sci.* **91**, 251–278.

JACOBSON, W. (1950). The action of folic acid antagonists on normal and leukaemic cells. *Proceedings of the International Society of Haematology*, 1950, p. 336. London: Heinemann.

JACOBSON, W. (1951). Biology of cell division. In *Transactions of the 13th Conference on Ageing*, pp. 155–194. New York: Josiah Macy Jr. Foundation.

JACOBSON, W. (1952). The role of the *Leuconostoc citrovorum* Factor (LCF) in cell division and the mode of action of folic acid antagonists on normal and leukaemic cells. *J. Path. Bact.* **64**, 245–247.

JACOBSON, W. (1953). A folic acid derivative functioning during cell division. *J. Physiol.* **122**, 40–41*P*.

JACOBSON, W. & WEBB, M. (1950). The two types of nucleic acid during mitosis. *J. Physiol.* **112**, 2–4*P*.

JACOBSON, W. & WEBB, M. (1952*a*). Nucleoproteins and cell division. *Endeavour*, **9**, 200–207.

JACOBSON, W. & WEBB, M. (1952*b*). The two types of nucleoproteins during mitosis. *Exp. Cell Res.* **3**, 163–183.

JUKES, T. H. & STOCKSTAD, E. L. R. (1948). Pteroylglutamic acid and related compounds. *Physiol. Rev.* **28**, 51–106.

PETERING, H. G. (1952). Folic acid antagonists. *Physiol. Rev.* **32**, 197–213.

SEEGER, D. R., COSULICH, D. B., SMITH, J. M. Jr. & HULTQUIST, M. E. (1949). Analogues of pteroylglutamic acid III, 4-amino-derivatives. *J. Amer. chem. Soc.* **71**, 1753–1758.

SUBBAROW, Y. (1947). Folic acid, ed. SUBBAROW, Y. *Ann. N.Y. Acad. Sci.* **48**, 257–349.

WOODS, D. D. (1953). Folic acid and related compounds in the metabolism of micro-organisms. *Brit. med. Bull.* **9**, 122–125.

WOOLLEY, D. W. (1950). Antimetabolites, ed. WOOLLEY, D. W. *Ann. N.Y. Acad. Sci.* **52**, 1336–1378.

EXPLANATION OF PLATE

PLATE 1

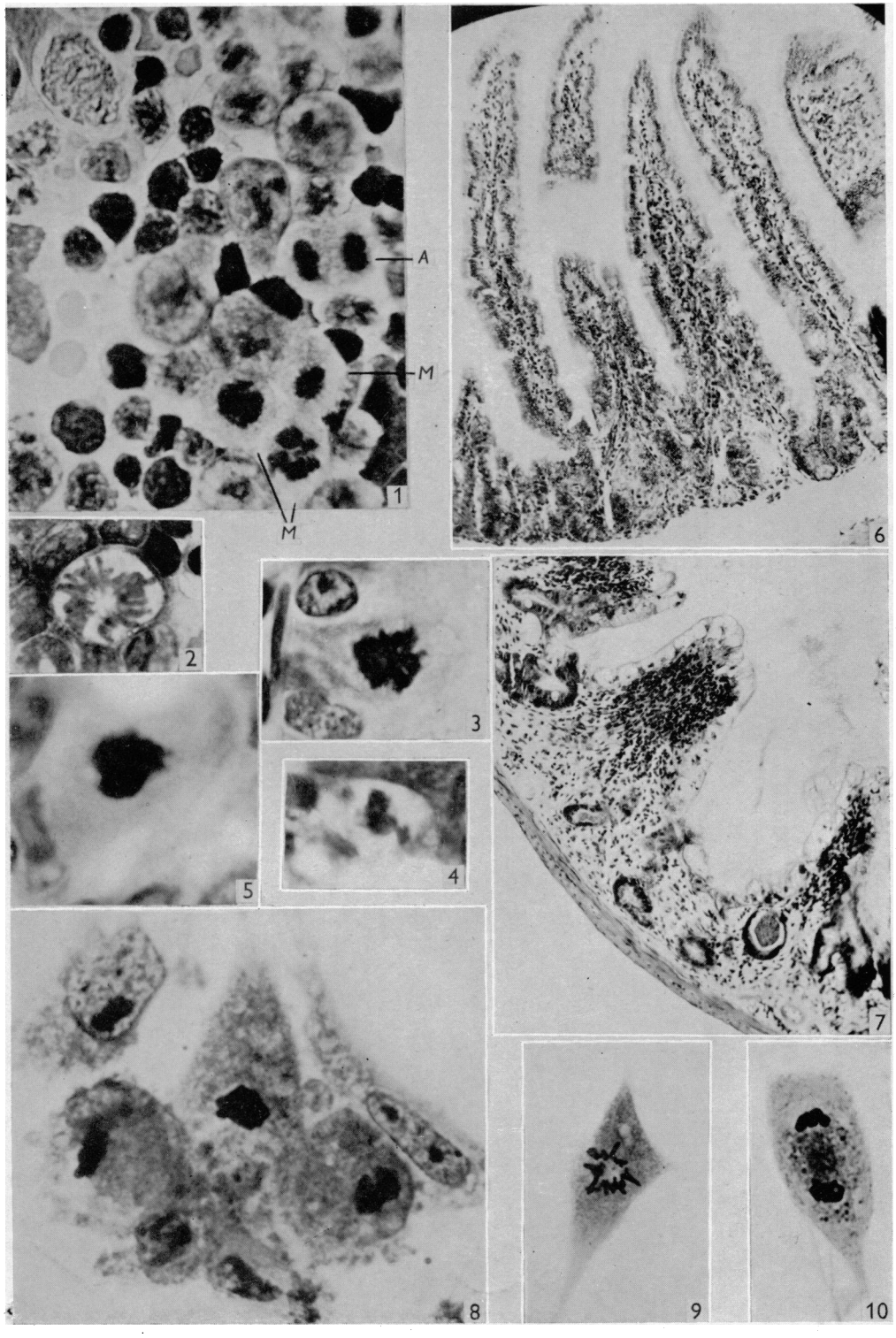
Fig. 1. Bone marrow of a leukaemic mouse 2 hr after subcutaneous injection of 1 mg aminopterin. In the left upper corner is a prophase; *M*, metaphase; *A*, anaphase, of leukaemic cells. Note the clumping of the chromosomes in metaphase. ×915. Smear stained with May-Grunwald and Giemsa.

Fig. 2. (Control to fig. 1.) A leukaemic cell in metaphase, showing the normal arrangement of chromosomes, from an untreated leukaemic mouse. ×915. Smear stained as in fig. 1.

Fig. 3. (Control to figs. 4 and 5.) A normal metaphase of an epithelial cell in a gland of the small intestine of the mouse. ×1100. Haematoxylin and eosin.

Fig. 4. Metaphase with clumped chromosomes in a gland of the small intestine of the mouse, 2 hr after the subcutaneous injection of 1 mg aminopterin. ×1100. Stained as in fig. 3.

Fig. 5. Large cell in metaphase with clumped chromosomes, from a gland of the small intestine of a mouse, 3 days after the subcutaneous injection of 0·2 mg aminopterin. ×1100. Stained as in fig. 3.



To face p. 616

Fig. 6. (Control to fig. 7.) Small intestine of an untreated mouse. × 135. Stained as in fig. 3.

Fig. 7. Small intestine of a mouse, 3 days after the subcutaneous injection of 0·2 mg aminopterin. Note the distorted glands and the large epithelial cells covering the shortened villi. × 135. Stained as in fig. 3.

Fig. 8. A group of dividing cells in a 48-hr-old tissue culture of chick embryo osteoblasts, fixed 15 min after exposure to aminopterin 1 : 2000. Left top: a cell in early prophase with a large nucleolus and chromosomes within the nuclear membrane. Centre: three cells in metaphase with tightly clumped chromosomes. In the lower part of the group is a telophase, the two daughter cells being formed. × 1100. May-Grunwald and Giemsa.

Fig. 9. (Control to fig. 8.) A normal metaphase in an untreated 48 hr tissue culture of chick embryo osteoblasts. × 825. Stained as in fig. 8.

Fig. 10. A normal anaphase in a 48 hr tissue culture of chick embryo osteoblasts. × 1100. Stained as in fig. 8. (Figs. 9 and 10 from Jacobson & Webb 1952*b*.)