injuries, because (1) the increased occurrence of binucleate lymphocytes appears to be related to exposure to exceptionally small amounts of radiation, and (2) lymphocytes may readily be obtained for examination. Hence examination for the presence of these cells serves as a practicable and sensitive adjunct to physical monitoring when circumstances indicate the desirability of biological monitoring.

Observation of an occasional lymphocyte of this type in irradiated animals and man has been reported by other investigators (*5, 6*). Quantitative estimates of pre- and post-exposure incidence, however, had not been made in these instances; hence it has not been possible to evaluate the significance of radiation exposure as regards the appearance of the cells. Unpublished reports suggest, however, that the cells are observed not uncommonly in radiation workers in other institutions—specifically in a "hazard group" at Los Alamos (*7*) and among luminizers in England (*8*). In both cases, exposure has apparently been kept below the current tolerance level.

It is likely that the increased incidence of lymphocytes with bilobed nuclei in the blood of cyclotron personnel during the early days of cyclotron operation actually indicates exposure to very small amounts of radiation, although physical monitoring at that time did not identify the nature and extent of exposure, and in fact has consistently indicated that exposure is well below tolerance. The supposition that the increased incidence of binucleate lymphocytes represents an effect of irradiation is supported by previously published experimental results (*2*) in which dogs, after a long control period, were exposed three times, 30 min each time, in positions approximating those reached by the two machinists described above. After each exposure there was a marked increase of lymphocytes with bilobed nuclei, followed by a gradual return to approximately normal levels during the period of approximately 2 months between exposures.

Lymphocytes with bilobed nuclei may appear in increased numbers in conditions other than exposure to ionizing radiation, as, for example, in infectious processes and in leukemia. The occurrence of an occasional cell of this type in the controls may reflect the normal incidence of low-grade infections in the group as a whole. It should be mentioned that the general health of the group remained good throughout the period under consideration. In particular, there was no indication of an unusual number of infections to explain the observed increased incidence of lymphocytes with bilobed nuclei during the first few months of cyclotron operation.

Since the occurrence of lymphocytes with bilobed nuclei remained low after completion of the protective dike, the detailed examination of peroxidase-stained smears is currently carried out only for new employees or when there is some definite change in operating procedure (e.g., reversal of the beam) or some question of personnel exposure. In such instances, the growing group of control observations and the individual's own control (new-hire) data are of great value.

References

1. INGRAM, M., and BARNES, S. W. *Phys. Rev.*, **75**, 1765 (1949).
2. ———. *Science*, **113**, 32 (1951).
3. MIESCHER, G. *Strahlentherapie*, **61**, 4 (1938).
4. FOGG, L. C., and WARREN, S. *Science*, **91**, 528 (1940).
5. GOODFELLOW, D. R. *Acta Radiol.*, **31**, 1 (1936).
6. JACOBSON, L. O., MARKS, E. K., and LORENZ, E. *Radiology*, **52**, 371 (1949).
7. CARTER, R. E. Personal communication.
8. BROWNING, E. Personal communication.

Manuscript received June 16, 1952.

A Quantitative Study of Folic Acid Requirements and Reversal of Aminopterin Inhibition in *Drosophila*[1,2]

Taylor Hinton

Department of Zoology,
University of California, Los Angeles

Studies reported by Goldsmith *et al.* (*1*) regarding the response of *Drosophila* to folic acid antagonists made use of a medium for growing the larvae which contained live yeast. This precludes the collection of exact quantitative data. The present report is concerned with results obtained (Table 1) with Drosophila reared on a chemically defined medium (*2*) and under aseptic conditions. With these last-named parameters controlled, the effect on growth of very precise amounts of a test substance can be determined.

Using the Oregon-R wild type strain of *D. melano-*

TABLE 1

Base medium (mg/ml)			(gamma/ml)
L-alanine	1.085	Biotin	0.020
L-arginine	0.794	B_{12}	0.04
L-aspartic acid	1.221	Ca-pantothenate	6.0
L-cystine	0.480	Choline chloride	20.0
L-glutamic acid	4.418	Pyridoxine	3.0
Glycine	2.328	Riboflavin	2.4
L-histidine	0.484	Thiamine	1.5
L-hydroxyproline	0.384	Niacinamide	10.0
L-isoleucine	1.260		
L-leucine	2.345		(mg/ml)
L-lysine	1.337	$MnSO_4 \cdot 4H_2O$	0.246
L-methionine	0.339	$MgSO_4 \cdot 7H_2O$	0.0129
L-phenylalanine	1.008	$FeSO_4$	0.0129
L-proline	1.682	KH_2PO_4	0.606
DL-threonine	1.512	K_2HPO_4	0.606
L-tryptophane	1.745	Thymine	0.004
L-tyrosine	1.240	NaCl	0.0129
L-valine	1.355	$CaCl_2$	0.0129
Sucrose	7.5	Agar	15.0
Cholesterol	0.1	Ribonucleic acid	1.0
Ergosterol	1.0	Inosine	0.25

[1] This investigation was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] G. W. Kidder, of Amherst College, kindly furnished the analogs used.

TABLE 2

TWO DOSE-RESPONSE TESTS TO PTEROYLGLUTAMIC ACID

PGA (milligamma/ml)	Original No. larvae		% Larvae to pupate		Av time in days to pupation		% Pupae to become adults	
	#1	#2	#1	#2	#1	#2	#1	#2
6000.00	15	19	80.0	100.0	10.7	10.4	100.0	100.0
300.00	12	17	50.0	100.0	11.2	10.8	100.0	100.0
150.00	12	17	58.3	94.1	11.0	10.9	100.0	100.0
75.00	9	—	55.6	—	11.8	—	80.0	—
30.00	13	8	46.2	100.0	11.7	11.6	33.3	75.0
15.00	18	10	50.0	100.0	14.3	12.5	0.0	20.0
7.50	15	10	20.0	60.0	14.3	13.5	33.3	0.0
3.00	15	10	13.3	30.0	16.0	15.2	0.0	0.0
1.50	14	—	0.0	—	—	—	0.0	—
0.75	16	18	0.0	0.0	—	—	0.0	0.0
0.15	18	9	0.0	0.0	—	—	0.0	0.0
0.00	9	8	0.0	0.0	—	—	0.0	0.0

gaster, two dose-response tests to folic acid (pteroylglutamic acid, PGA) were carried out to determine the minimal and optimal amounts necessary for growth (Table 2). The minimal amount of PGA that will support any development beyond the larval stage was determined to be between 1.5 and 3.0 milligamma/ml medium; the optimal was the highest concentration used (6000 milligamma/ml), but 30 milligamma/ml may be considered to give only slightly suboptimal development.

A preliminary investigation was made of several of the analogs of PGA to determine their effectiveness in the replacement of PGA. Tests were run on synthetic folinic acid (citrovorum factor), teropterin (pteroyltriglutamic acid, PTGA), and aminopterin (4-aminopteroylglutamic acid). The first two were found to be capable of replacing PGA; the latter inhibited development (Table 3).

On the basis of molecular weight, amounts were used of PTGA and of the citrovorum factor comparable to 75 milligamma/ml of PGA. At least in the amounts used, neither the citrovorum factor nor PTGA were quite as effective in fulfilling the growth requirements of Drosophila, using time to pupation as the criterion, although the difference does not appear to be significant. Higher concentrations of citrovorum and PTGA were not tested.

TABLE 3

ATTEMPTS TO REPLACE PTEROYLGLUTAMIC ACID

	Original No. larvae	% Larvae to pupate	Av time in days to pupation	% Pupae to become adults
PGA (75 milligamma/ml)	68	92.6	11.7	96.8
PGA (6000 milligamma/ml)	41	82.9	10.8	91.2
Citrovorum (10 u/ml)	53	96.2	12.6	100.0
PTGA (122 milligamma/ml)	66	98.9	12.1	100.0
Aminopterin (3 milligamma/ml)	19	0.0	—	0.0

TABLE 4

AMINOPTERIN INHIBITION RELEASED BY VARIOUS AMOUNTS OF PGA
(Aminopterin, 3 milligamma/ml)

PGA (milligamma/ml)	Original No. larvae	% Larvae to pupate	Av time in days to pupation	% Pupae to become adults
9,600.0	24	87.5	10.8	100.0
8,200.0	25	96.0	10.9	87.5
4,800.0	26	100.0	10.7	92.3
2,880.0	28	85.7	10.9	100.0
480.0	23	91.3	10.9	100.0
144.0	26	92.3	11.3	95.8
82.0	20	85.0	11.9	70.6
14.4	24	0.0	—	0.0

Since the substitution of aminopterin gave no growth, it was of interest to see if it actually inhibited or could not be utilized by the organism. Therefore, various concentrations of aminopterin were put into the medium in the presence of a slightly suboptimal concentration of PGA (30 milligamma/ml). All concentrations of aminopterin prevented development. Only the control with no aminopterin grew. The concentrations of aminopterin tested were, in milligamma/ml: 3, 100, 300, 750, 1500, and 3000, with an average of 24.5 original larvae at each concentration. It could therefore be concluded that the action of aminopterin is that of an antagonist to PGA.

Studies were then made of the ability of PGA to release the inhibition caused by aminopterin. For this purpose, a small amount of aminopterin (3 milligamma/ml, shown to be inhibitory in the previous experiment) and increasing amounts of PGA were used. The results (Table 4) show that PGA is able to release the inhibition, in part, when it is present in a concentration as low as 82.0 milligamma/ml

TABLE 5

INCREASING AMOUNTS OF AMINOPTERIN IN THE PRESENCE OF 6000 MILLIGAMMA PGA/ML

Aminopterin (milligamma/ml)	Original No. larvae	% Larvae to pupate	Av time in days to pupation	% Pupae to become adults
0	10	100.0	11.2	90.0
3	21	100.0	11.0	100.0
100	20	95.0	11.5	100.0
1000	18	11.1	16.0	0.0

and to release it completely at 480.0 milligamma/ml.

This experiment showed that the inhibition caused by a small amount of aminopterin could be released by a slightly suboptimal amount of PGA. It was decided next to see how much aminopterin could be used without an inhibiting effect in the presence of an optimal amount of PGA. The results of these tests are presented in Table 5.

The results presented in Table 5 show that amounts of aminopterin as high as 100 milligamma/ml can be used without showing marked inhibition if the amount of PGA is also high. Even when 1000 milligamma of aminopterin/ml medium was used, complete inhibition was not achieved in the presence of 6000 milligamma PGA.

An inhibition index can be calculated which is quite consistent if one takes the concentration of inhibitor necessary to produce only half maximum growth (50% pupation), and obtains the ratio of it to the concentration of the metabolite used. In Table 4, it can be seen that 50% pupation occurs somewhere between a concentration of PGA of 14.4 and 82.0 milligamma/ml when the concentration of aminopterin is 3. Assuming that 50% pupation would occur with roughly 35 milligamma/ml PGA, the ratio of inhibitor to metabolite would be 3/35, or 0.086. If the same treatment is given to the data in Table 5, the ratio is 500/6000, or 0.083. These two values are remarkably close, indicating a constant relative inhibitive effect of aminopterin to the concentration of PGA.

Using a chemically defined medium in the absence of microorganisms, the quantitative results are of a different order of magnitude. This is strikingly illustrated by comparing the concentrations used by Goldsmith *et al.* (*1*) in a yeast medium with the concentrations found adequate in the present work on a synthetic medium. To obtain complete inhibition by aminopterin, the former workers had to use 75,000 to 200,000 milligamma/ml, whereas in the present work, 3 milligamma/ml was shown to be completely inhibitory. Nevertheless, the two sets of data agree qualitatively.

References

1. GOLDSMITH, E. D., HARNLY, M. H., and TOBIAS, E. B. *Ann. N. Y. Acad. Sci.*, **52**, 1342 (1950).
2. HINTON, T., NOYES, D. T., and ELLIS, J. *Physiol. Zoöl.*, **24**, 335 (1951).

Manuscript received June 16, 1952.

Reversal of *p*-Aminobenzoic Acid Inhibition of Growth of Rickettsiae by *p*-Hydroxybenzoic Acid in Agar-Slant Tissue Culture

N. Takemori and M. Kitaoka

National Institute of Health of Japan, Tokyo

The rickettsiostatic action of *p*-aminobenzoic acid (PABA) was first observed in mice by Snyder, Maier, and Anderson (*1*) in 1942 and also in the chick embryo by Greiff, Pinkerton, and Moragues (*2*) in 1944. Independently, Takemori (*3*) found in 1944 (published in 1949) the inhibitory action of PABA on the growth of typhus rickettsiae in tissue culture. It was argued that "if the mode of action of PABA on typhus rickettsiae be similar to that of the action of the sulfonamides on sulfonamide-sensitive organisms, it is suggested that an 'essential metabolite' will be found for rickettsiae which reverses the inhibitory action of PABA on typhus rickettsiae and may be chemically related to PABA" (*3*).

Following the announcement of *p*-hydroxybenzoic acid (POB) as a bacterial vitamin required by a mutant of *Escherichia coli* (*4*), the mechanism of inhibition of *E. coli* by PABA was shown to depend on competition with POB (*5*). The competition between POB and PABA observed with *E. coli* has led to similar reversal studies with POB of the rickettsiostatic action of PABA in chick embryos and mice (*6*).

In view of the rickettsiostatic action of PABA observed in tissue culture by Takemori (*3*), it has seemed desirable to investigate whether this action of PABA is also reversed in *in vitro* growth tests by POB. The present study shows that POB is able to reverse the rickettsiostatic action of PABA in agar-slant tissue culture.

The Wilmington strain of murine typhus (*Rickettsia typhi*), Smith strain of Rocky Mountain spotted fever (*R. rickettsii*),[1] M.K. strain of rickettsialpox (*R. akari*),[1] Nine Mile strain of Q fever (*Coxiella burnetii*),[1] and 6BC strain of psittacosis virus[1] which were employed in these experiments have been maintained on Zinsser's agar-slant culture (*7*) with dark embryonic tissue. All these agents grew quite satisfactorily on this medium, and passages were made at 8–15-day intervals.

The medium was prepared as described previously for the cultivation of rickettsiae and the viruses of lymphogranuloma venereum and tick-borne encephalitis (*8*). A 3.5% agar solution is made in twice-distilled water, dissolved at 15 psi for 15 min, cooled, and kept at about 55° C. To 75 ml of a double-strength Tyrode solution are added 50 ml of horse serum and 4 ml of a 0.04% solution of phenol red. This Tyrode-serum mixture is filtered through a Seitz filter, warmed to 55° C, and mixed with 75 ml of 3.5%

[1] Obtained through the courtesy of Rocky Mountain Laboratory, R. J. Huebner, H. R. Cox, and K. F. Meyer, respectively.