hypoalbuminemia and hyper-gammaglobulinemia may also occur in other vitamin deficiencies, such as niacin.

Summary. The plasma or serum proteins of rhesus monkeys maintained on various vitamin deficient diets were studied by chemical and by electrophoretic analysis. Partial or complete deficiency of vit. B_6 resulted in hypoalbuminemia and a significant increase in the gamma globulin component. On a niacin deficient diet 2 monkeys developed a lowered albumin and an increase in the gamma globulin. A low choline diet led to a decrease in the albumin and to an increase in the beta globulin. The addition of 1% cholesterol to the diet of control or vit. B_6 deficient monkeys produced a significant increase in the alpha-3 component. The electrophoretic composition of the plasma did not appear to be altered by a state of early chronic scurvy. The mobilities of the plasma components appeared to be increased by an ascorbic acid deficient diet but were unaffected by the other regimens.

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Effects of Purine and Pyrimidine Analogues on Development of *Rana pipiens* (19402)

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Analogues of the purine and pyrimidine constituents of the nucleic acids have been found to have a variety of biological effects which may be ascribed to various types and degrees of interference with the biosynthesis of nucleic acids and related compounds(1).

The desirability of testing such antimetabolites on a variety of biological species and systems has previously been mentioned(1) for such studies contribute both to the comparative biochemistry of nucleic acid metabolism and to the knowledge of the mode of action of the antimetabolites. The present report is concerned primarily with an extension of the antimetabolite studies to the problems of chemical embryology. Although the effects

TABLE I. Inhibition of Development of *Rana pipiens* by Purine and Pyrimidine Analogues.

Substance	Earliest stage inhibited	Min effective conc., mg/100 ml
8-Azaadenine	8	7.5
6-Mercaptopurine	8–9	20
5-(2′, 4′-Dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine	8	5
2-Amino-5-(4′-chlorophenoxy)-4-hydroxypyrimidine	8–9	10
5-(4′-Chlorophenyl)-2,4-diaminopyrimidine	12–13	2.5
5-(4′-Chlorophenyl)-2,4-diamino-6-ethylpyrimidine	14–15	5
2,4-Diamino-5-(3′,4′-dichlorophenyl) pyrimidine	20	10
6-Amyl-5-(4′-chlorophenyl)-2,4-diaminopyrimidine	23	10
5-(4′-Chlorophenoxy)-2,4-diaminopyrimidine	13	10
5-(4′-Chlorophenoxy)-2,4-diamino-6-methylpyrimidine	14	10
2,4-Diamino-5-phenoxypyrimidine	16–17	10
5-(3′4′-Dichlorophenoxy)-2,4-diaminopyrimidine	21	10
5-Benzyl-2,4-diamino-6-methylpyrimidine	14–15	20
5-(4′-Chlorobenzyl)-2,4-diamino-6-methylpyrimidine	17–18	20
2,4-Diamino-5-(4′-nitrobenzyl)-6-methylpyrimidine	21	20
2,4-Diamino-5-(3′,4′-dimethoxybenzyl) pyrimidine	21	20
4-Amino-pteroylglutamic acid (Aminopterin)	12–13	25
8-Azaguanine	25+	10
5-Nitrouracil	25+	10
5-Bromouracil	25+	10
5-Hydroxyuracil	25+	10
2,4-Diamino-5,6-dimethylpyrimidine	23+	20
2,6-Diaminopurine	25+	50
Xanthopterin	*	50
2,4-Diamino-6,7-dimethylpteridine	*	15

* Not inhibitory at concentrations tested.

of some antimetabolites on the development of the chick have been studied, except for a brief attempt by Brachet(2) and a more recent preliminary report(3) amphibia have not been utilized for such studies. The amphibian egg lends itself readily to experimental and biochemical observations on early morphogenesis. The present investigation is concerned with the development of *Rana pipiens* embryos from fertilization to Shumway stage 25(4).

Methods. Eggs were obtained from female *Rana pipiens* by induced ovulation and were fertilized by a suspension of macerated frog testes in spring water. The twenty-five analogues were tested by immersing embryos chosen at the two cell stage in eight different concentrations of the compound (in the range of 1 to 25 mg per 100 ml) made up in spring water. Fifty embryos, 25 per finger bowl, were used for each concentration. The bowls were kept in an incubating refrigerator at 18-19°C during the course of the experiment. Readings of the developmental progress were taken at regular intervals and compared with those of appropriate controls.

The *results* are expressed in Table I in terms of the earliest stage of development which is inhibited by the analogue and the minimal effective concentration to produce complete inhibition at this point. At lower concentrations the development is inhibited at critical stages (8,9,12,13,15,16,20-23) but eventually passes through each stage, and between critical points development proceeds at essentially a normal rate even in the presence of the inhibitor. Thus certain substances which inhibit at an early stage may, at low concentrations, show retardation of development at several rather specific stages. In general it may be stated that the substances affecting the earliest stages of development are those which are found to affect purine metabolism primarily, in microbiological studies (5); those which inhibit at neurulation are, in general, antagonists of folic acid or citrovorum factor in the microbiological system (6,7). Embryos demonstrating inhibition at the later periods of development possess abnormalities such as reduction of head structures, failure to hatch, abnormal gill and opercular development, reduction in embryo

size and other abnormalities indicative of interference with normal development.

The results of a more complete study under way(8), including histological, histochemical and reversal attempts, support the view that these effects reflect the interference with purine and pyrimidine utilization by the embryo rather than being due to generalized toxic reaction.

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A Technic for Determination of Adrenocorticotrophin in Blood.* (19403)

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The oxycellulose technic of Astwood *et al.* (1) for the isolation of adrenocorticotrophin (ACTH) from pituitary has been successfully adapted to the quantitative analysis of ACTH in rat blood.

Methods. Collection of blood. Male rats of the Sprague-Dawley strain, weighing 150 to 300 g, were employed. Each rat was anesthetized with ether and bled from the abdominal aorta into a heparinized syringe. Four volumes of glacial acetic acid were immediately added to the sample and the mixture was thoroughly stirred. Pools of blood-glacial acetic acid mixtures were processed for ACTH as described below.

Technic for concentration of blood ACTH. 1) The mixture of blood and glacial acetic acid is gently stirred at 70-75°C for 30 minutes in a water bath and then transferred to a large pyrex cylinder. 2) To each ml of the above mixture are added 13.0 ml distilled water with constant stirring. 3) 0.1 g Hyflo-Super-cel per ml of blood is stirred into the mixture which is then allowed to stand for 24 hours. The Super-cel settles to the bottom of the cylinder. 4) The supernatant is siphoned into a pyrex cylinder for further processing; the Super-cel is discarded. 5) A slurry of prepared† oxycellulose‡ (0.04 g per ml blood) is added to the supernatant with continuous stirring; stirring is continued for 24 hours to allow for complete "adsorption" of ACTH on oxycellulose. 6) The oxycellulose is now allowed to settle for 24 hours. 7) The supernatant is siphoned off and discarded; the oxycellulose is collected on a course, sintered-glass funnel and washed with 30-ml quantities of 0.1 N acetic acid until the washings are

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† A weighed amount of oxycellulose is washed into a sintered-glass funnel with three 25-ml quantities of 0.1 N acetic acid, then washed successively with 25 ml distilled water, 25 ml 0.1 N HCl, 25 ml distilled water and, finally, with three 25-ml quantities 0.1 N acetic acid. A slurry of the washed oxycellulose is prepared with 15 to 30 ml 0.1 N acetic acid.

‡ Samples of oxidized cellulose (11-12% COOH) were generously supplied by the Research Laboratories, Tennessee Eastman Co.