The morphology and biologic behavior of the 8-azaguanine-resistant subline grown in dba strain mice receiving 8-azaguanine resembles the sensitive control line grown in antagonist-free mice. At 9 days, in addition to growth of lymphomatous tissue subcutaneously, there results a florid leukemia with severe infiltration into lymph nodes, spleen and liver, leukocytosis with escape of lymphoblasts into the blood and a severe secondary anemia. The mean survival time of mice bearing the resistant and dependent cells and given 8-azaguanine is strikingly similar to the control line. Table III gives the comparative blood picture of dba mice growing the sensitive and resistant leukemic cells, the histologic pattern of infiltration and a comparison of the mean survival times of these groups.

It has been assumed by Kidder and coworkers(9) that neoplasms, unlike normal tissues, require guanine for growth and that 8-azaguanine acts as a competitive inhibitor of this changed metabolism of guanine by cancerous tissues. Results have been equivocal, however, relating to the question as to whether or not free guanine is metabolized by cancerous tissues in contradistinction to normal tissues(16-18). It is hoped that the variant leukemic cells described here, which are resistant to and partially dependent on 8-azaguanine for growth, will offer more suitable material for studies on the role of guanine in cancer.

Summary. (1) The development of resistance in a transplantable acute lymphocytic leukemia of the mouse to a guanine analog, 8-azaguanine, is reported. (2) Optimal growth of the resistant cells is attained in mice receiving near maximum tolerable doses of 8-azaguanine, indicating partial dependence of the variant line on the analog used in developing resistance. (3) The sensitive (control) line of leukemia has shown nearly complete inhibition of tumor growth at comparable transfer generations. (4) A-methopterin and TEM inhibit growth of the resistant line. (5) The biologic behavior of resistant leukemic cells, grown in mice receiving 8-azaguanine, strikingly resembles sensitive cells grown in antagonist-free mice: A florid leukemia develops with leukocytosis and escape of lymphoblasts into the blood, severe infiltration into lymph nodes, spleen and liver, resulting in death at approximately the same time as controls.

16. Brown, G. B., Roll, P. M., Plentl, A. A., and Cavalieri, L. F., *J. Biol. Chem.*, 1948, v172, 469.

17. Brown, G. B., Bendich, A., Roll, P. M., and Sugiura, K., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 501.

18. Graff, S., Engelman, M., Gillespie, H. B., and Graff, A. M., *Cancer Res.*, 1951, v11, 388.

Received September 21, 1951. P.S.E.B.M., 1951, v78.

Protein Metabolism of Amphibian Embryo. III. Incorporation of Methionine into Protein of Gastrulae.* (19119)

RICHARD M. EAKIN, PHYLLIS B. KUTSKY, AND WILLIAM E. BERG.

From the Department of Zoology, University of California, Berkeley.

An increasing number of studies is being made of regional differences in the physiology of the amphibian gastrula which may eventually elucidate the general problem of embryonic induction and, specifically, the mechanism whereby dorsal mesoderm (organizer of Spemann) evokes the differentiation of neural tissue from ectoderm. Friedberg and Eakin(1) showed that C^{14} of glycine is more rapidly incorporated into protein or intermediary compounds by the dorsal halves of gastrulae and neurulae than by the ventral

* Supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

1. Friedberg, F., and Eakin, R. M., *J. Exp. Zool.*, 1949, v110, 33.

TABLE I. Incorporation of Methionine S^{35} by Dorsal and Ventral Half-Gastrulae.

Series	Exp.		No. half embryos	Dosage: mg methionine	Dosage: μc S^{35}	Radioactivity of protein methionine: cts/min/μ mol sulfur D*	cts/min/μ mol sulfur V*	% activity D	% activity V
I	*Rana pipiens*	1	200	.64	3.2	64	51	56	44
		2	150	.64	2.8	56	41	58	42
		3a	140	.65	2.5	92	80	54	46
		3b	140	.65	2.5	117	70	63	37
		4a	150	.70	2.4	78	69	53	47
		4b	150	.70	2.4	95	72	57	43
II	*Hyla regilla*	5	200	.70	2.9	68	63	52	48
		6	200	.61	2.1	65	62	51	49
		7	200	.70	2.0	77	60	56	44

* D = dorsal; V = ventral.

halves. A similar study is here reported on the incorporation of methionine labelled with S^{35}.

For each experiment early gastrulae, stage 10 of Shumway(2), of *Rana pipiens* or of *Hyla regilla* were removed from the membranes and bisected frontally along the animal-vegetative axis; the dorsal and ventral halves were separately collected and incubated in about 3 ml of sterile Holtfreter's solution containing 0.2 mg/ml radioactive methionine (Table I). The methionine was obtained from Abbott Laboratories, Chicago. As in the glycine study(1), efforts were made to prevent microorganisms utilizing the amino acid. The culture flasks were attached to open Warburg manometers and gently rocked in a water bath at 20°C for 5 hours. After incubation boiling 1M sodium acetate buffer at pH 4.5 was added to the flasks, the contents boiled, cooled and homogenized in a Potter type, ground glass homogenizer. The residue from centrifugation was washed at least 6 times with 10 ml aliquots of the acetate buffer. Ten mg of carrier dl-methionine was included in either the first or second wash aliquot. The residual protein was hydrolyzed with 25 ml of 8N HCl by autoclaving for 3½ hours at 15 lb. The general procedure outlined by Simpson and Tarver(3) was followed for the separation of methionine from other sulfur-containing amino acids, the conversion of methionine sulfur to sulfate, and subsequent determination of radioactivity and standard base equivalence of a benzidine sulfate precipitate. The data have been expressed in terms of specific activity, counts/min./μmole of sulfur. Self-absorption corrections have been made where necessary.

The results of 6 experiments with *R. pipiens* and 3 with *H. regilla* are shown in Table I. In every instance the dorsal halves had a higher specific activity. In *Rana* the average difference between the activities of the two halves was 14%, in *Hyla* 6%. Using a statistical method† for analyzing 4 paired measurements in *Rana*, the sigma of the difference of the means was 1.65 ($P < 0.01$). In experiments 3 and 4 the duplicate runs were averaged.

The methionine used in these experiments contained other radioactive compounds, namely: methionine sulfoxide and traces of homocystine and cystine, as shown by radioautographs of paper chromatograms of the fresh culture medium. In some experiments radioautographs were also made from chromatograms of the homogenate supernatant, of the hydrolyzed protein, and of the methionine fraction. No radioactive compound was found which was not present in the fresh culture medium.

Other experiments (not given in Table I) showed that, as in the glycine study(1), whole gastrulae had a much lower uptake of the amino acid than those bisected and that controls (gastrular halves boiled prior to incuba-

2. Shumway, W., *Anat. Rec.*, 1940, v78, 139.

3. Simpson, M. V., and Tarver, H., *Arch. Biochem.*, 1950, v25, 384.

† $\sigma d_M = \sqrt{\frac{Mx^2 - M^2x}{(n-1)}}$ where x = the difference between the members of the individual pairs.

tion or halves to which acetate buffer was added immediately after they were placed in the culture flasks) exhibited much less activity. Whole or cut embryos allowed to develop after 5 hours incubation in the culture medium suffered no apparent ill-effects of the treatment.

This study augments the evidence that the dorsal half of the amphibian gastrula has a greater physiological activity than the ventral half and specifically a higher rate of protein metabolism. Several studies by Brachet and recently that of Steinert(4) suggest a relationship between ribonucleoprotein and neural induction. The curve shown by the latter for the synthesis of ribonucleic acid resembles that which we obtained in our study of the uptake of glycine, a precursor of purine. We do not know whether the greater incorporation of the sulfur-bearing amino acid, methionine, by the dorsal half-gastrula than by the ventral half has peculiar significance. It is not impossible that this difference is indirectly related to the apparent importance of sulfhydryl groups in the induction of the neural plate by the organizer as shown by Rapkine and Brachet(5) and Lallier(6).

Summary. The dorsal half of the amphibian gastrula was shown to have a significantly greater incorporation of methionine into protein than the ventral half.

4. Steinert, M., *Bull. Soc. Chim. Biol.*, 1951, v33, 549.

5. Rapkine, L., and Brachet, J., *Bull. Soc. Chim. Biol.*, 1951, v33, 427.

6. Lallier, R., *Bull. Soc. Chim. Biol.*, 1951, v33, 439.

Received September 24, 1951. P.S.E.B.M., 1951, v78.

Renal Excretion of Digitoxin in the Rabbit and Dog.* (19120)

SHIRLEY ST. GEORGE, RENÉ BINE, JR., MEYER FRIEDMAN, AND CATHARINE BLAND.
From the Mount Zion Hospital, San Francisco, Calif.

Although the renal excretion of digitoxin was found to be negligible in the rat(1), the normal human subject appeared to excrete as much as 40% of a given dose of the drug(2). In an effort to discover whether the rat handled digitoxin anomalously in this particular respect, the possible renal excretion of the drug was studied in two other species. The results of this study are here reported.

Methods. Three rabbits weighing an average of 2525 g were given 0.2 μg of digitoxin per g of weight by intravenous injection. The urines of these rabbits then were collected for 24 hours. The total urine volume was extracted for its digitoxin content according to previously described methods(1,2). The assay of the extract was performed with the embryonic duck heart preparation(1,2). Two male dogs weighing 9 and 10 kg respectively, were given 0.2 μg of digitoxin per g of body weight. A third dog weighing 15 kg was given 0.1 μg of digitoxin per g of body weight. The urine of each of the 3 dogs was collected for 24 and 48 hours. A 200 cc sample of each 24-hour urine was extracted and assayed exactly as described(2) for human urine. The extraction and sensitivity of the assay were such that a minimum of 10 μg could be detected and assayed in a 24-hour urine collection.

Results. The kidney of the rabbit does not appear to excrete digitoxin. Thus, although each of the 3 rabbits received approximately 500 μg of digitoxin by vein and each exhibited a good urine flow, none of the urine collections contained detectable amounts of digitoxin. This indicates that less than 0.5% of a given dose of digitoxin is excreted in an unchanged form by the rabbit kidney. It is of course

* Aided by grants from The Life Insurance Medical Research Fund, Eli Lilly and Co., and the U. S. Public Health Service.

1. Friedman, M., Bine, R., Jr., and Byers, S. O., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 406.

2. Friedman, M., Bine, R., Jr., Byers, S. O., and Bland, C., *Circulation*, 1950, v2, 749.