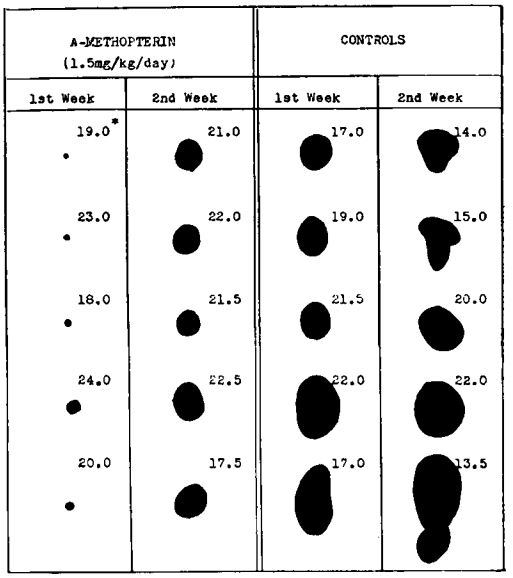

FIG. 1.
Area diagrams of tumors in mice treated with A-Methopterin compared with untreated controls.
* Weight of mouse in grams.

similar effect; however, marked inhibition could be demonstrated only when the drug was administered in amounts which killed a large per cent of the mice. A-Methopterin appears to exhibit a more favorable therapeutic index. It has been outstanding in its effect among numerous compounds which might interfere with the utilization of folic acid or possibly with the synthesis of nucleic acid. In fact, it has been the most effective compound of over 700 miscellaneous compounds that have been screened. It is therefore advanced as a compound worthy of further experimental study and for clinical trial in various types of cancer.

Summary. The compound 4-Amino-N^{10}-methyl pteroylglutamic acid, has shown the ability to inhibit the growth of the transplantable mouse Sarcoma 180 when administered intraperitoneally in concentrations of 1.5 mg/kg each day for 7 days. At this dosage very few mice died from drug toxicity. The inhibition is most apparent when the compound is administered early in the development of the tumor.

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Beneficial Effects of Liver on Growth and Survival in the Immature Hyperthyroid Mouse.*

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Available data indicate that desiccated whole liver will prolong survival and counteract the growth retardation of immature rats fed massive doses of thyroactive materials.[1-5] The protective factor is distinct from any of the known nutrients and is retained in the water-insoluble fraction of liver.[3-5] The present experiment was undertaken to determine the effects of liver and liver fractions on growth and survival in the immature hyperthyroid mouse.

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[2] Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition*, 1947, **34**, 431.

[3] Ershoff, B. H., *Arch. Biochem.*, 1947, **15**, 365.

[4] Ershoff, B. H., *Exp. Med. Surg.*, 1948, **6**, 438.

[5] Ershoff, B. H., and McWilliams, H. B., *Science*, 1948, **108**, 632.

Procedure and results. One hundred and eighty-seven male mice of the Webster strain were selected for the present experiment at 21 to 23 days of age and an average weight of 10.7 g. The basal ration employed consisted of sucrose 53%, casein† 30%, cottonseed oil 10%, Sure's Salt Mixture No. 1[6] 5%, and cellulose‡ 2%. To each kg of the above diet were added the following synthetic vitamins: thiamine hydrochloride 20 mg, riboflavin 20 mg, pyridoxine hydrochloride 20 mg, nicotinic acid 60 mg, calcium pantothenate 60 mg, biotin 4 mg, folic acid 10 mg, p-aminobenzoic acid 200 mg, inositol 400 mg, 2-methyl-naphthoquinone 10 mg, and choline chloride 2 g. Each mouse also received once weekly a vitamin A-D concentrate§ containing 25 U.S.P. units of vitamin A and 2.5 U.S.P. units of vitamin D together with 1.5 mg of α-tocopherol acetate. The following supplements were incorporated in the basal ration in place of an equal amount of sucrose: (1) 10% yeast‖ (2) 10% whole liver powder¶ (3) 10% extracted liver residue,** consisting of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances and (4) 2% of Wilson's liver concentrate 1-20,†† containing the water-extractable material of raw liver. Each of the above diets was in turn supplemented with U.S.P. desiccated thyroid,‡‡ thyroxin,§§ or iodinated casein,‖‖ added in place of an equal amount of sucrose. These were incorporated in the above rations at the following levels: thyroid, 0.5%; thyroxin, 50 mg/kg of diet, and iodinated casein, 0.2%. Animals were placed in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib.* Feeding was continued for 15 days or until death, whichever occurred sooner. Animals that died or lost weight during the first 5 days of feeding were discarded and not included in the tabulation of data. Results are summarized in Table I.

The findings indicate the response of the immature mouse is similar to that of the rat when fed massive doses of thyroactive materials. The addition of desiccated thyroid, thyroxin or iodinated casein to the basal ration resulted in a marked retardation of growth which was completely counteracted by the administration of whole liver powder or extracted liver residue. Liver concentrate 1-20 failed to promote growth in animals fed desiccated thyroid or iodinated casein; it did exert a growth-promoting effect, however, in animals fed thyroxin. Yeast was ineffective in promoting growth on any of the diets employed. Data on survival correspond to those obtained in the rat. Less than 50% of the mice fed the basal ration supplemented with desiccated thyroid, thyroxin or iodinated casein survived the experimental period of 15 days. The incidence of survival was significantly increased on diets containing whole liver powder or extracted liver residue. Yeast and liver concentrate 1-20 were considerably less active than whole liver or extracted liver residue in prolonging survival in the hyperthyroid mouse.

In the present experiment whole liver powder prolonged survival and counteracted the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin, or iodinated casein. The protective factor(s) was apparently distinct from any of the known nutrients and was retained in the water-insoluble extracted liver residue. Bosshardt *et al.*[7] have recently found that the

† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

[6] Sure, B., *J. Nutrition*, 1941, **22**, 449.

‡ Ruffex, Fisher Scientific Co., St. Louis, Mo.

§ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per g.

‖ Brewers' Type Yeast No. 200, Anheuser-Busch, Inc., St. Louis, Mo.

¶ Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

** Extracted Liver Residue, Wilson Laboratories, Chicago, Ill.

†† Liver Concentrate Powder 1-20, Wilson Laboratories, Chicago, Ill.

‡‡ Thyroid Powder, U.S.P., Armour and Co., Chicago, Ill.

§§ Thyroxin (Synthetic Cryst.), Roche-Organon, Inc., Nutley, N. J. The material was dissolved in .1 N NaOH, adjusted to pH of 8.0, and diluted to a volume containing 50 mg in 12.5 cc.

‖‖ Protamone, Cerophyl Laboratories, Kansas City, Mo.

TABLE I.
Comparative Effects of Liver and Yeast on Growth and Survival of Immature Mice Fed Massive Doses of Thyroactive Substances.

Supplement to basal ration	Thyroactive supplement	No. of animals	Initial body wt, g	Gain in body wt on following days of exp. 10th, g	Gain in body wt on following days of exp. 15th, g	% surviving*
0	0	12	10.6	6.2 (12)	8.1 (12)	100.0
0	Thyroid	15	10.6	2.6 (5)	5.4 (1)	6.7
10% yeast	"	10	10.7	2.9 (8)	5.2 (6)	60.0
10% whole liver powder	"	14	10.5	7.7 (14)	11.2 (13)	92.9
10% ext. liver residue	"	15	10.4	6.6 (15)	10.0 (15)	100.0
1% liver conc. 1-20	"	15	10.5	3.3 (9)	3.8 (6)	40.0
0	Iodinated casein	12	10.9	2.8 (10)	4.2 (4)	33.3
10% yeast	" "	10	11.0	2.8 (10)	5.1 (6)	60.0
10% whole liver powder	" "	12	10.7	8.4 (11)	11.9 (11)	91.7
10% ext. liver residue	" "	9	11.1	7.3 (8)	9.7 (8)	88.9
1% liver conc. 1-20	" "	8	11.1	2.0 (8)	3.6 (6)	75.0
0	Thyroxin	11	10.7	1.0 (10)	2.6 (5)	45.5
10% yeast	"	9	10.8	3.6 (6)	4.3 (6)	66.7
10% whole liver powder	"	11	10.8	5.3 (11)	8.4 (9)	81.8
10% ext. liver residue	"	12	10.6	8.9 (11)	12.8 (11)	91.7
1% liver conc. 1-20	"	12	10.9	6.4 (7)	9.8 (6)	50.0

The values in parentheses indicate the number of animals which survived and on which averages are based.

* Experimental period 15 days.

growth retardation of immature mice fed massive doses of iodinated casein (protamone) could be counteracted by the administration of Wilson's liver concentrate 1-20 and other water-soluble extracts of liver rich in "animal protein factor" or vitamin B_{12}. Under conditions of the present experiment, Wilson's liver concentrate 1-20 contained virtually no activity in regard to either growth or survival for immature mice fed massive doses of iodinated casein or desiccated thyroid. It did exert a growth-promoting effect, however, in immature mice fed thyroxin, although gain in body weight was less than that obtained with extracted liver residue. The cause of this discrepancy is not readily apparent. Yeast appears to be less beneficial to the hyperthyroid mouse than to the hyperthyroid rat. Previous findings indicate that yeast is as effective as whole liver powder in prolonging survival of immature rats fed massive doses of desiccated thyroid;[3] such does not appear to be the case for the immature thyroid-fed mouse.

Recent findings by Bosshardt *et al.*,[7] Register *et al.*,[8] and Nichol *et al.*,[9] indicate that requirements for vitamin B_{12} are increased in the hyperthyroid animal. Since liver concentrate 1-20 (which contains vita-

[7] Bosshardt, D. K., Paul, W. J., O'Doherty, K., Huff, J. W., and Barnes, R. H., *J. Nutrition*, 1949, **37**, 21.

min B_{12}) failed to counteract the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin or iodinated casein while extracted liver residue was as potent as whole liver powder in this regard, it appears that extracted liver residue contains one or more factors other than vitamin B_{12} which are also required in increased amounts by the hyperthyroid mouse. These findings are in agreement with those previously reported for the rat.[4] When animals on purified rations are made hyperthyroid, a multiple dietary deficiency may be precipitated. Whether the first limiting factor will be a deficiency of vitamin B_{12}, of the antithyrotoxic factor(s) of extracted liver residue or of still other nutrients appears to depend on such factors as the pre-test dietary regime, the composition of the diet employed, bacterial synthesis or strain and species differences in nutritional requirements.

Summary. Whole liver powder prolonged survival and counteracted the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin or iodinated casein. The protective factor(s) was retained in the water-insoluble fraction of liver.

8 Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 129.

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An Evaluation of Respiratory Depression by Alveolar Gas Changes During Pentothal Sodium Anesthesia.*

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Recent studies of intravenous anesthetic agents, notably pentothal sodium, have placed renewed emphasis on quantitative analyses of physiological changes occurring during anesthesia. The work of Gruber,[1] Gruhzit, Dox, Rowe and Dodd,[2] Beecher and Moyer,[3,4] Moyer,[5] and most recently McCann,[6] contain pneumographic tracings which illustrate the characteristic respiratory patterns produced by intravenous anesthetic agents. Beecher and Moyer[4] have correlated these qualitative studies with minute volume determinations and intermittent blood levels of oxygen and carbon dioxide. Etsten and Himwich[7,8] intensively investigated the reflex responses under pentothal anesthesia and have set forth criteria for determining the stages of pentothal anesthesia. Previous studies have employed

* Work done under contract with the Air Materiel Command, Wright Field.

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1 Gruber, C. M., *J. Pharm. and Exp. Therap.*, 1937, **60**, 143.

2 Gruhzit, O. M., Dox, A. W., Rowe, L. W., and Dodd, M. C., *J. Pharm. and Exp. Therap.*, 1937, **60**, 125.

3 Beecher, H. K., and Moyer, C. A., *J. Clin. Invest.*, 1941, **20**, 549.

4 Moyer, C. A., and Beecher, H. K., *J. Clin. Invest.*, 1942, **21**, 429.

5 Moyer, C. A., *J. Thoracic Surg.*, 1941, **11**, 131.

6 McCann, J. C., *Current Res. in Anesth.-Anal.*, 1947, **26**, 89.

7 Himwich, H. E., and Etsten, B., *J. Nerv. and Mental Disease*, 1946, **104**, 407.

8 Etsten, B., and Himwich, H. E., *Anesthesiology*, 1946, **7**, 536.