6 hours) paralleled that produced by cortical hormone. The percentage of circulating eosinophiles dropped from 14.3 to 2.4, the per cent of lymphocytes remaining unaltered.

The absolute number of eosinophiles dropped from 2700 to 400 cell/mm^3. In 2 adrenalectomized trichinous mice which received epinephrine, the total count had doubled within 6 hours. The percentages of eosinophiles and the other types of cells remained the same.

Urethane in aqueous solution (1 mg or ½ mg per g body weight) was injected into 6 intact trichinous mice. After 6 hours the absolute number of lymphocytes had dropped to approximately half, the eosinophiles to less than half the initial levels. Much greater decreases were obtained in 6 mice which survived 5 or 6 daily anesthetic doses of urethane. The percentage of eosinophiles dropped from 9.3 to 2.3, the absolute number from 1800 to 100. The absolute number of lymphocytes dropped from 12600 to 2200.

Urethane-induced lymphopoenia has been observed in adrenalectomized as well as intact rats and mice.[4,5] Urethane depressed the white blood cell count in mouse myeloid leukemia, but adrenal cortical extract was ineffective.[6] These observations indicate that the effect of urethane on normal blood lymphocytes and leukemic myeloid cells is not mediated by the adrenal cortex. In the present experiments eosinopoenia paralleled lymphopoenia following the injection of urethane. Adrenalectomized trichinous mice did not tolerate anesthetic doses of urethane, and in these experiments it could not be determined whether depression of blood eosinophiles occurs as an effect of urethane in the absence of the adrenal cortex. Spink[7] demonstrated that reduction in the eosinophile count of trichinous guinea pigs occurred with a superimposed bacterial infection. The eosinophile count was also suppressed in a patient developing bacterial infection during the course of trichinosis.

Summary. The number of circulating eosinophiles of trichinous mice was decidedly reduced by the administration of cortical hormone. The same effect was induced by epinephrine in intact, but not in adrenalectomized, trichinous mice. Sex steroids in the doses used did not significantly reduce the percentage of circulating eosinophiles in 4 intact and 2 adrenalectomized trichinous animals. Eosinopoenia was induced by urethane in trichinous mice. Whether or not urethane-induced eosinopoenia is mediated by the adrenal cortex has not been determined.

[4] Dury, A., and Robin, E. D., *Endocrinology*, 1948, **42**, 320.

[5] Lu, C. S., Thesis, University of Minnesota, 1947.

[6] Lu, C. S., Kirschbaum, A., *Proc. Internat. Cancer Res. Congress*, 1947, 105.

[7] Spink, W. W., *Arch. Int. Med.*, 1943, **54**, 805.

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17143. Effect of Pteroylglutamic Acid and Vitamin B_{12} on Growth of Rous Tumor Implants.

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Earlier work from this laboratory showed that pteroylglutamic acid was essential for the growth of implants of Rous sarcoma in young chicks.[1] The effect of other dietary factors was much less pronounced.[2] A search was then made for additional dietary factors which might affect the growth of this sarcoma in chicks.

Day-old chicks in groups of 10 were fed

[1] Little, P. A., Sampath, A., Paganelli, V., Locke, E., and SubbaRow, Y., *Tr. N. Y. Acad. Sci.*, Series II, 1948, **10**, 91.

[2] Little, P. A., Oleson, J. J., and SubbaRow, Y., *J. Lab. and Clin. Med.*, 1948, **33**, 1139.

TABLE I.
Comparative Effects of Pteroylglutamic Acid, Liver Extract and Vitamin B_{12} on the Growth of Rous Sarcoma.

Supplements	Amt inj. daily	No. of tests	Total No. of chicks	% chicks w. tumors	Avg tumor size
None	—	6	60	3	+
Pteroylglutamic Acid	5 γ	3	23	61	+
" "	10 γ	5	50	78	++
" "	50 γ	6	57	88	+++
15 Unit Liver	.01 cc	1	10	70	+
15 " "	.025 cc	2	20	60	+
15 " "	.05 cc	2	20	60	++
15 " "	.10 cc	2	20	85	+++
Vit. B_{12} concentrate	≅ .05 γ	2	20	55	+
" " "	≅ 0.1 γ	1	10	60	++
" " "	≅ 0.5 γ	2	20	75	+++
" " "	≅ 1.0 γ	2	19	68	+++
" " "	≅ 2.0 γ	2	20	85	+++
Vit. B_{12} + Pteroylglutamic Acid	≅ 0.5 γ + 10 γ	1	10	90	++++
Vit. B_{12} + Pteroylglutamic Acid	≅ 2.0 γ + 10 γ	1	10	100	++++

the synthetic pteroylglutamic acid deficient diet and water *ad libitum* and were injected with tumor suspension as previously described.[2] Supplements of pteroylglutamic acid and the preparations reported here were injected intramuscularly daily at the levels indicated in the table.

It was found that 15 unit liver extract* would apparently replace pteroylglutamic acid at levels as low as 0.01 ml per day in stimulating the growth of Rous sarcoma in chicks fed this synthetic diet. It is known that parenteral liver extracts contain very little pteroylglutamic acid[3] and therefore cannot substitute for this factor in the nutrition of chicks. A preliminary study was then made of the properties of the factor in liver which was responsible for tumor stimulation.

Since 15 unit liver extract (beef) is a rich source of vitamin B_{12} and the properties of the liver factor were found to be similar to this vitamin, highly purified concentrates of vitamin B_{12} were also tested. These were found to give the same results as the liver extract itself. The preparations were previously assayed microbiologically† and by a chick growth method[4] for their vitamin B_{12} content.

Some typical results are presented in Table I. Pteroylglutamic acid alone gives a high incidence of growing tumors of good size. The liver extract alone gives quite similar results. The effect of the liver is duplicated by dosages of vitamin B_{12} as low as 0.05 γ per day. The higher levels of this factor give larger sized tumors than the lowest levels in the 14-day test period. When pteroylglutamic acid and vitamin B_{12} are given together, the tumors come up earlier and attain greater size than with corresponding levels of either factor alone.

Thus it appears that vitamin B_{12} does not entirely replace pteroylglutamic acid in its growth promoting effect on the Rous tumor but is required in addition to give maximum tumor growth under these particular dietary conditions.

Summary. Vitamin B_{12} has been demonstrated to have a role in the growth of Rous sarcoma in chickens comparable to that previously shown for pteroylglutamic acid.

* 15 Unit liver-Lederle (prepared from beef liver).

3 Clark, G. W., *Am. J. Med. Sci.*, 1945, **209**, 520.

† We thank Mr. A. C. Dornbush for these assays.

4 Whitehill, A. R., Wieland, O., and Oleson, J. J., unpublished data.

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