Another factor which may affect the virus yield is the rate of adsorption of virus to host cell. Delbruck(7) concluded that the yield of virus particles in broth cultures would be increased by a decrease in adsorption rate for two reasons: the loss of liberated virus by multiple adsorption onto unlysed bacteria would be lessened, and the bacterial population would reach a higher total figure because delayed adsorption would give more time for bacterial growth. Since the adsorption rate of T4 is about 20 times that of T5, this difference may well contribute to the better yields obtainable with phage T5. The rate of adsorption of T4 is in part determined by the salt concentration in the medium(8). An attempt to further increase the yield of phage T4 by reducing the salt concentration to decrease the adsorption rate did not result in a significant gain in phage production.

Summary. Some of the variables involved in the production of high titer phage stocks by the agar layer method have been investigated using coli phage T4r as an example. The significant factors have been shown to be the number of virus particles and the number of bacteria inoculated per plate, the length of incubation, the volume of soft agar in the agar layer and the volume of broth used for extraction of the virus from the agar. By an appropriate adjustment of these variable factors it is possible to obtain stocks of the various coli phages of the T series ranging from 10^{11} to 10^{12} infectious particles/ml.

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Received April 27, 1951. P.S.E.B.M., 1951, v78.

Therapeutic Activity of Xanthopterin Added to p-Aminobenzoylglutamic Acid in Experimental Macrocytic Anemia of Rat.* (19077)

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We have reported(1) the results of experiments on rats in which macrocytic anemia was induced by a folic acid-deficient diet containing sulfaguanidine as an inhibitor of the intestinal flora. These animals were injected with the following substances separately: Folic acid, vitamin B_{12}, xanthopterin, cobalt ions and a mixture of xanthopterin and p-aminobenzoylglutamic acid. It was found that simultaneous administration of the last 2 drugs did not prevent the death of rats showing a severe vitamin deficiency. However, rats receiving streptomycin, instead of sulfaguanidine, and given the xanthopterin-paraminobenzoylglutamic acid mixture, showed a clear improvement of the blood picture.

In the present experiments we attempted to obtain a chronic folic acid deficiency in the rat by adding streptomycin, as an inhibitor of intestinal flora, to the usual synthetic diets. In addition the activity of xanthopterin, of p-aminobenzoylglutamic acid, and of both substances administered together was tested.

Experimental procedure. A total of 50 male albino rats, from this Institute strain, weighing 40 g each, were used. The animals were fed *ad libitum* with a standard folic acid-deficient diet(2). One percent of streptomycin was added to the diet as inhibitor of intestinal flora, instead of sulfasuxidine. The symptoms of deficiency were studied according to the technic previously described(1).

* We are indebted to Lepetit Laboratories-Milano for supplying p-aminobenzoylglutamic acid, and to F. Hoffmann La Roche-Basel for xanthopterin used in these studies.

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2. Black, Mc Kibbin, and Elvehjem, PROC. SOC. EXP. BIOL. AND MED., 1941, v47, 308.

TABLE I.*

Groups	R.B.C. × 10^6 /cu mm	W.B.C. × 10^3 /cu mm	Hgb % (Sahli)	C. I.
		Before treatment		
I	4.4 ± .42	8.5 ± 1.07	83 ± 9	1.52 ± .002
II	4.6 ± .63	8.8 ± 1	86 ± 9.6	1.40 ± .01
III	4.3 ± .05	8.9 ± .77	86 ± 4.80	1.67 ± .14
IV	4.4 ± .39	8.5 ± .34	94 ± 6.24	1.81 ± .01
		After treatment		
I	7.8 ± .22	13.6 ± 1.80	82 ± 2	.90 ± .001
II	5.3 ± .93	9.1 ± .77	91 ± 10	1.48 ± .01
III	5.7 ± .26	8.8 ± .94	88 ± 5.12	1.29 ± .09
IV	7.8 ± .02	11.2 ± .06	97 ± 9.05	1.07 ± .54

C.I. = Color index. Stand. error = $\sqrt{\frac{S\,y^2}{n(n-1)}}$.

* Emmens, C. W., *Principles of Biological Assay*, Chapman and Hall, London, 1948, p17.

Immediately after a period of depletion (about 180 days), the animals showed a hematological picture characteristic of folic acid deficiency. Twenty were selected and divided by random selection into 4 groups, on which the therapeutic activity of the following substances, administered parenterally (g/wt/animal) was tested: Group 1, pteroylglutamic acid (folic acid), 200 μg; Group 2, p-aminobenzoylglutamic acid, 300 μg; Group 3, xanthopterin, 200 μg; Group 4, xanthopterin, 200 μg + p-aminobenzoylglutamic acid, 300 μg.

Results and discussion. The results are shown in Table I and Fig. 1 to 4. The deficient diet containing 1% streptomycin causes folic acid deficiency in about 50% of the animals. This technic has the following advantages: the animals show a more regular growth curve and a greater resistance to infection and the well known antithyroidal action of sulfa drugs is avoided. Rats fed on the streptomycin-containing diet develop a hematological picture equally characteristic but less severe than that shown by animals fed with diets containing sulfaguanidine. Erythrocyte levels range from 4 to 5 millions per cmm, and the color index is above normal. The hematological data do not show significant variations even when observations were continued beyond 180 days. During this period definite evidence of vitamin deficiency appeared and persisted for 300 days.† Persistence of the anemia suggests that the animals were maintained in a chronic but partial deficiency state. Small amounts of folic acid may have been synthesized in the intestine in amounts which prevented severe bone marrow damage and death of the animals. It is possible, therefore, that streptomycin does not completely inhibit folic acid synthesis. Thus the addition of streptomycin to the diet seems to be particularly useful in the study of chronic folic acid deficiency in the rat. With this technic it is possible to avoid the periodic addition of suboptimal doses of folic acid to the diet as Kodiceck and Carpenter(3) have suggested. The experiment with the first group of rats treated with 200 μg of folic acid was performed to determine whether the hematological picture was due exclusively to the chronic deficiency of the vitamin. The results obtained (Fig. 1) confirm this hypothesis. We have used these results, therefore, in evaluating the activity of the other substances tested. The structural formulae of the substances used is given. It is evident that all of them contain a nucleus which appears also in the folic acid molecule.

The observations shown in Fig. 3 reveal (a) that administration of xanthopterin causes some increase in number of red blood cells, but is unable to restore the number to normal, (b) that the color index remains high while the leukocyte counts remain almost unchanged. Xanthopterin has very little activity,

† Personal data unpublished.

3. Kodiceck and Carpenter, *Blood*, 1950, v6, 499.

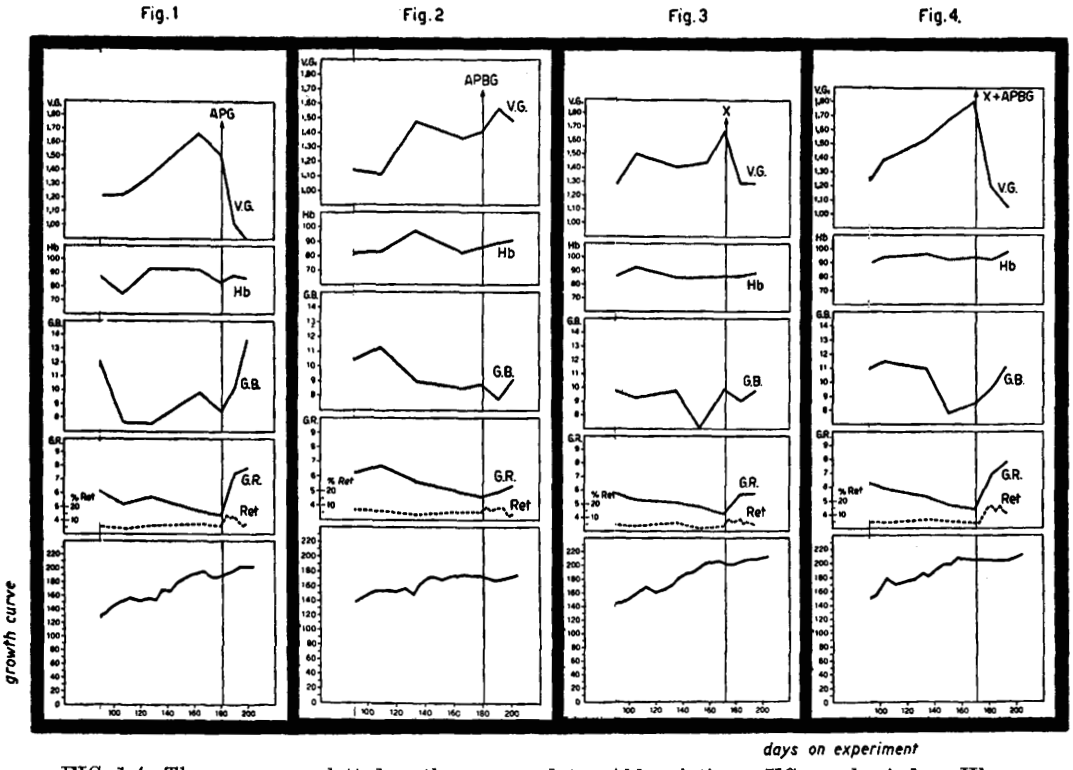
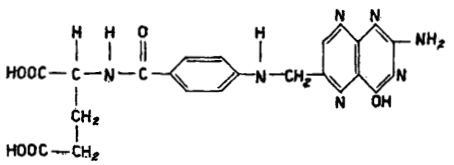
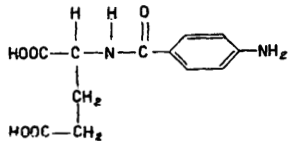


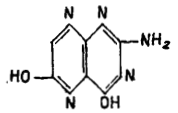
FIG. 1-4. The curves are plotted on the average data. Abbreviations: VG = color index; Hb = hemoglobin % (Sahli); Ret. = reticulocytes %; GB = white blood cells 10^3/cu mm; GR = red blood cells 10^6/cu mm; APG = pteroylglutamic acid; APBG = p-aminobenzoylglutamic acid; X = xanthopterin; X + APBG = xanthopterin + p-aminobenzoylglutamic acid.



Pteroylglutamic acid (folic acid).



P-aminobenzoylglutamic acid.



Xanthopterin.

therefore, causing some improvement in the anemia but not modifying its characteristic picture or altering the leukopenia. P-aminobenzoylglutamic acid had no activity (Fig. 2): the hematological findings were not altered by its administration. The mixture of xanthopterin-paraminobenzoylglutamic acid had complete folic acid-like activity. Although its action was somewhat slower than that of folic acid, the hematological picture eventually returned to normal (Fig. 4). Reticulocytosis occurred as with folic acid administration. While a reticulocyte crisis does not occur in animals treated with xanthopterin alone or with p-aminobenzoylglutamic acid alone, such a crisis appears in groups of animals receiving folic acid and the mixture of xanthopterin-paraminobenzoyl-glutamic acid. This observation suggests that these animals could perform folic acid synthesis if administered the two main components of its molecule.

Conclusions. (1) Chronic folic acid deficiency was induced in rats with a highly purified diet containing 1% streptomycin. (2) Xanthopterin causes a small increase in the number of erythrocytes but does not alter the color index or leukopenia. (3) P-aminobenzoylglutamic acid has no effect on the hematological picture resulting from a

chronic deficiency of folic acid in the rat. (4) The simultaneous parenteral administration of amounts of xanthopterin and p-aminobenzoylglutamic acid in the same molecular ratio existing in folic acid, has a complete and specific therapeutic activity almost as rapid as that of folic acid. (5) This indicates that rats can synthesize folic acid from simultaneously administered xanthopterin and p-aminobenzoylglutamic acid.

Received June 4, 1951. P.S.E.B.M., 1951, v78.

Changes in Brain Water, Brain Chloride, and Serum Chloride after Bilateral Nephrectomy in Rats. (19078)

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In 11% of the 138 cases of cerebral edema reviewed by the authors from the autopsy files at the New Haven Hospital(1), nephritis and renal failure were found to be associated with edema of the brain. Other scattered references in the literature indicate that uremia and renal disease are often associated with cerebral swelling and edema(2-5). Hence one might expect to find edema in the brains of nephrectomized rats. The observations of Stewart-Wallace(6) suggest that chloride concentration, which he found much higher in edematous than in normal brain tissue, might serve as a biochemical index of cerebral edema. It is to be expected that in edema, in which there is an increase in the extracellar phase of a tissue, there should also be an increase in chloride, the predominant extracellular ion.

Although methods exist for the determination of tissue chlorides(7-9) preliminary experiments made it clear that a special procedure for chloride determination in small quantities of brain tissue is desirable. Enough normal and nephrectomized rats were used to justify statistical analysis of the results. Serum chloride and brain water determinations were done on both.

Experimental. Rats of the Sprague-Dawley and Yale strains were used. Bilateral nephrectomy was carried out under nembutal anesthesia and care was taken to leave the adrenal glands undisturbed. The animals were housed in an air conditioned animal room in which temperature and humidity were maintained at 75°C., and 45-55%, respectively. Purina laboratory feed and water were supplied *ad libitum*. The animals survived for 3 days after the operation, and just before death they were anesthetized with nembutal and a sample of blood was removed under oil by cardiac puncture. The skull was then removed with an electric hand saw, the pia-arachnoid was reflected, and the brain was lifted out with a spatula. The animals lived until the brain was removed. The blood covering the surface of the brain was removed by blotting with filter paper. The brain was then bisected and each half was weighed quickly. One half of the brain was used for the chloride determinations and the other for the water determinations.

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