chicken embryos by yolk sac inoculation for a total of 28 consecutive passages.

After the second yolk sac passage 0.5 ml of a 10% yolk sac suspension was inoculated into the right sinus of each of 2 normal turkeys. The sinuses became swollen 5 and 8 days after inoculation respectively. Sinus exudate aspirated on the first day after the appearance of clinical symptoms was bacteriologically sterile and killed all of 10 chicken embryos 6 to 12 days after inoculation into the yolk sac. Clinical sinusitis was produced in a total of 10 additional turkeys with yolk sac material from the 3rd, 4th, and 16th egg passages respectively.

Filtration studies on the egg-adapted strain of the agent of turkey sinusitis were carried out using a 1/100 dilution of infected yolk sacs. At least 50.0 ml amounts of the suspension were filtered through Berkefeld V, Berkefeld N, and Seitz E K filters respectively. The filtrates obtained, together with the unfiltered suspension, were tested for infectivity by inoculation into groups of 15 embryonating eggs. All embryos inoculated with the unfiltered suspension and the Berkefeld V filtrate died 4 to 8 days after inoculation. However, all embryos inoculated with Berkefeld N and Seitz E K filtrates respectively were still living 14 days after inoculation. It is evident that the causal agent passed through a Berkefeld V filter but did not pass readily Berkefeld N or Seitz E K filters.

During the first 10 egg passages microscopic examination of impression smears of infected yolk sacs stained by Machiavello's technic revealed only an occasional red staining coccoid body. However, beginning with the 14th egg passage when the infective titer of yolk sacs reached 10^{-8} or more small, coccoid bodies were regularly observed both intra- and extracellularly. These bodies were found to vary in size from approximately 0.5 μ to 1.0 μ or more in diameter and were frequently imbedded in a matrix. The bodies described above closely resembled morphologically the bodies found in similar impression smears of yolk sacs infected with the etiological agents of the lymphogranuloma-psittacosis group (*Chlamydozoaceae*).

Further studies on the characteristics of the agent of turkey sinusitis and its possible etiological relationship to an agent isolated from air sac infection in turkeys by Minard and Jungherr[8] are in progress.

Summary. 1. An agent capable of producing clinical sinusitis in turkeys was isolated and propagated in the yolk sac of the developing chicken embryo. 2. Impression smears of infected yolk sacs stained by Machiavello's technic revealed morphologic forms closely resembling those found in the lymphogranuloma-psittacosis group of agents (*Chlamydozoaceae*).

[8] Minard, E. L., and Jungherr, E., 1944, Proc. 16th An. Conf. Lab. Workers in Pullorum Dis. Control, Coll. of Agr., Univ. of Conn., mimeog. report.

16320

Observations on the Effect of 4-Amino-Pteroylglutamic Acid on Mice.

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The production of a syndrome in mice by feeding a crude "antagonist" of pteroylglutamic acid (PGA) and its prevention by raising the dietary level of PGA was described.[1] The syndrome was marked by slow growth followed by a loss in weight, low hemoglobin and low white blood cell count. Recently, another substance, "4-amino" pteroylglutamic acid ("4-amino-PGA"), which is strongly

[1] Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 368.

antagonistic to PGA in the *Streptococcus fecalis* R test, was described.[2] This compound differs from PGA only in having an amino group in place of the hydroxyl group on the pteridine ring. Its inhibition of the growth of *S. fecalis* R was reversed by increasing the level of PGA. The effect of the compound in the nutrition of mice was studied in the present investigation.

Experimental. The animals received a purified diet without PGA and with 1% succinylsulfathiazole, identical with the diet previously described[1] except that *p*-aminobenzoic acid was omitted in Experiment 2. In a preliminary experiment it was found that mice died within a few days after being placed on a diet containing levels of 1 mg of 4-amino-PGA or more per kilo. Accordingly in Experiment 1 low levels of 4-amino-PGA were fed with various amounts of PGA ranging up to comparatively high levels. The results are in Table I. At a level of 0.3 mg the 4-amino compound had a slight tendency to depress the blood values which was reversed by high levels of PGA. When the level of the compound was raised to 1.0 mg, mortality was high even with large amounts of PGA.

In Experiment 2, the effects of PGA upon mice receiving 4-amino-PGA was explored further. Higher levels of 4-amino-PGA were used, ranging up to 3 mg per kilo of diet. Some reversal of the toxic effect was observed at the 0.3 mg level, but no protection was afforded by PGA when the 4-amino compound was fed at levels of 1 or 3 mg per kilo of diet (Table II).

No lesions were visible upon gross examination at autopsy.

TABLE I.

Effects of Various Levels of PGA and 4-Amino PGA on Growth and Hematology of Mice. Six Mice Were Used in Each Group.

Group	Supplement in mg per kilo of diet		Body wt and No. of survivors					Hemoglobin g per 100 cc		White cells ($\times 10^3$) per mm^3	
	PGA	4-amino PGA	0	1	2	4	5 wk	at 2 wk	at 4 wk	at 2 wk	at 4 wk
1	0.1	0	11	16	17	21 (5)	21 (5)	10.3	13.0	7.1	10.0
2	1.0	0	11	16	17	21	22 (6)	14.3	14.4	12.5	9.4
3	10	0	11	16	18	21	22 (6)	16.2	17.6	8.8	11.8
4	100	0	12	18	19	23	23 (6)	13.7	16.4	9.0	13.0
5	0.1	0.1	13	17 (5)	18	20	19 (5)	11.3	12.2	11.4	7.3
6	1.0	0.1	12	18	21	26	26 (6)	15.8	15.8	11.8	13.4
7	10	0.1	12	18	21	25 (5)	24 (4)	13.4	15.8	13.2	11.8
8	100	0.1	12	17	19	23	24 (6)	15.6	16.9	12.8	10.9
9	0.1	0.3	13	16	15 (5)	20	20 (5)	8.5	10.4	7.4	5.6
10	1.0	0.3	12	14 (5)	16	20	21 (5)	13.9	13.4	15.3	9.1
11	10	0.3	12	19	22	24	24 (6)	14.1	15.7	14.4	11.9
12	100	0.3	13	18	20	22	21 (6)	15.7	17.2	14.5	16.8
13	10	1.0	13	12 (3)	14 (1)	(0)	(0)	12.1	—	10.3	—
14	100	1.0	11	14 (2)	17 (2)	22 (2)	23 (2)	10.9	11.5	7.0	10.2

Summary. The 4-amino analogue of pteroylglutamic acid was fed to mice as a dietary supplement. There was some indication of reversal by pteroylglutamic acid of the effect on mice when the level of the antagonist was 0.3 part per million of diet. Death was found to occur within a few days with levels of 1 part per million of diet or higher. The effect was not reversed by feeding high levels

[2] Seeger, D. R., Smith, J. M., Jr., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1947, **69**, 2567.

TABLE II.
Effects of PGA and 4-Amino PGA on Growth and Survival of Mice in Experiment 2. Five mice were used per group.

Group	Supplement in mg per kg of diet		Body wt and (in parentheses) No. of survivors					Avg survival time in days*
	PGA	4-amino PGA	0	1	3	5	8 wk	
15	0.1	0	10	14	21	24	22 (5)	
16	1.0	0	10	13	19	19	19 (5)	
17	10	0	10	15	20	22	24 (5)	
18	100	0	11	13	20	22	24 (5)	
19	0.1	0.1	10	15	20	24	24 (5)	
20	1.0	0.1	10	16	21	26	28 (5)	
21	10	0.1	10	16	21	23	24 (5)	
22	100	0.1	11	16	23	26	30 (5)	
23	0.1	0.3	10	(0)				4
24	1.0	0.3	10	13 (4)	20	23	26 (4)	
25	10	0.3	10	13	18	20	21 (5)	
26	100	0.3	9	14	18 (4)	21	24 (4)	
27	0.1	1.0	10	9 (1)	(0)			5
28	1.0	1.0	10	13 (1)	(0)			8
29	10	1.0	10	9 (1)	(0)			7
30	100	1.0	10	(0)				5
31	0.1	3.0	10	(0)				6
32	1.0	3.0	11	(0)				4
33	10	3.0	10	(0)				4
34	100	3.0	10	(0)				4

* In the case of groups in which all the animals died during the experimental period.

of pteroylglutamic acid. This finding may be contrasted with the inhibitory effect of the 4-amino compound on *S. fecalis* R which is reversed by pteroylglutamic acid.

16321

Relation Between Induced Hyperthyroidism and an Unidentified Chick Growth Factor.*

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The use of biological assays for study of unidentified growth factors is often restricted by the extent to which they can measure low concentration of a factor in a given material. This may be due to the small response of the animal to a given supplement. A series of studies have shown that the chick requires an unidentified growth factor found in condensed fish solubles,[1] whole liver powder, and certain other liver fractions.[2,3] The increased growth rate of the chick as a result of adding supplements carrying the factor has been used as an assay in determining some of the prop-

* Published with the approval of the Director of the Wisconsin Experiment Station. Supported in part by grants from the Borden Company, New York City, and the Commercial Solvents Corporation, Terre Haute, Ind.

1 Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Science,* 1948, in press.

2 Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *Poultry Science,* 1948, in press.

3 Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *J. Biol. Chem.,* 1947, **170**, 419.