The inability of folic acid to protect the chick embryo from the toxic action of the "4-amino" PGA's, even when given in ratios of 1000 to 4000 to 1, is further evidence of the difficulty in protecting against or reversing the activity of these compounds. Franklin *et al.*[5] have obtained slight and limited protection against "4-amino" PGA with large doses of folic acid, and Hertz and Tullner,[15] using a ratio of folic acid to 4-amino pteroylglutamic acid of approximately 300 to 1, prevented the "anti-folic" inhibition of the chick oviduct response to estrogenic stimulation. In frogs, however, a folic acid to "4-amino" PGA ratio of 100 to 1 did not prevent the "4-amino" PGA inhibition of the oviduct response to estrogens.[16] It appears clear, therefore, that folic acid may prevent the toxic effects of the "4-amino" folic acids but the protective ratio necessary will undoubtedly differ quantitatively for the specific "anti-folic" compound and the biological system used.

The apparently specific action of 2,6-diaminopurine on blood formation merits further study. It is of interest that Bendich and Brown[17] have shown that 2,6-diaminopurine may serve as a precursor of nucleic acid guanine, an observation which cannot, as yet, be related to the mechanism of blood formation.

Summary. The "4-amino" pteroylglutamic acids profoundly inhibit the growth of the chick embryo, with the production of developmental abnormalities. These compounds are active in the range of 0.003 to 0.005 mg/egg, and their actions are not prevented by large doses of folic acid. The "4-amino" folic acids with aspartic acid, threonine and alanine substituted for the glutamic acid are considerably less toxic but produce similar toxicological effects. Other compounds allied to folic acid are relatively non-toxic and do not possess the growth-inhibitory activity of the "4-amino" compounds.

2,6-diaminopurine, at LD_{50} doses, does not seem to be an active growth inhibitor, but it produces a pale embryo, presumably deficient in hemoglobin.

[16] Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 299.

[17] Bendich, A., and Brown, G. B., *J. Biol. Chem.*, 1948, **176**, 1471.

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17221. Distribution of C'^4, Administered as CaC'^4O_3, in Rat Liver Glycogen and Blood Glucose and Lactate.*

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In prior studies[1-5] it has been shown that the distribution of isotopic carbon in rat liver glycogen following the feeding of a labeled compound may serve as an indicator of intermediary pathways. Under the proper experimental conditions, the distribution of isotope in blood glucose and lactate might reasonably be expected to mirror that in the liver glycogen. Should this expectation be realized, it would be possible to obtain information from the degradation of the carbohydrates from the blood of larger animals and man comparable

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[1] Wood, H. G., Lifson, N., and Lorber, V., *J. Biol. Chem.*, 1945, **159**, 475.

[2] Lifson, N., Lorber, V., Sakami, W., and Wood, H. G., *J. Biol. Chem.*, 1948, **176**, 1263.

[3] Lorber, V., Lifson, N., Wood, H. G., and Sakami, W., *Am. J. Physiol.*, 1948, **155**, 452.

[4] Shreeve, W. W., Feil, G. H., Lorber, V., and Wood, H. G., *J. Biol. Chem.*, 1949, **177**, 679.

[5] Lorber, V., Cook, M., and Bordeaux, J., *Fed. Proc.*, 1949, **8**, 99.

TABLE I.
Distribution of C'^4 in the Degradation Fractions from Rat Liver Glycogen, and Blood Glucose and Lactate.

Exp. No.	Animals wt in g	Total activity administered as CaC'^4O_3 in cts/min.	Activity of 7th day sample of respiratory CO_2 in cts/min/mg C	Activity of the various fractions from blood glucose + lactate, and liver glycogen, in cts/min./mg C				
				Blood glucose + lactate carbon atoms		Liver glycogen carbon atoms		
				3 and 4	1,2,5 and 6	3 and 4	2 and 5	1 and 6
1	328	5.6×10^6	—	24	2	8	0	
2	303	5.1×10^6	100	31	—	5	0	
3	293	2.6×10^7	380	35	0	44	0	1
4	329	2.3×10^7	640	88	0	65	0	3

in significance to that derived from the study of rat liver glycogen. The present paper reports some experiments designed to test this point in the rat.

Methods. Pellets of CaC'^4O_3[6] were implanted intraperitoneally in 4 unfasted white rats. After seven days on stock diet the animals were anaesthetized with intraperitoneal nembutal, and as much blood as possible withdrawn by syringe from the abdominal aorta. Bleeding was facilitated by administering warm Ringer's solution into the inferior vena cava by syringe. The diluted blood, rendered non-clotting by heparin, was placed in a stoppered flask, and held in a water bath at 38°C for 5 hours, with moderate agitation. At the end of this time, practically all the blood glucose was converted to lactate by the glycolytic enzymes of the blood. The blood was deproteinated with metaphosphoric acid, the lactate removed by continuous ether extraction, and degraded as previously described.[1] The acetaldehyde arising from the $KMnO_4$ oxidation of the lactate, representing carbons 1,2,5 and 6 of the glucose, was oxidized without further degradation. The liver glycogen was isolated and degraded by the "bacterial" degradation method described previously.[1] In experiments 1 and 2 the acetaldehyde arising from $KMnO_4$ oxidation of the lactate from the glycogen was precipitated and counted as the 2-4 dinitrophenylhydrazone. In experiments 3 and 4 the aldehyde was further degraded by the iodoform reaction. Daily collections of respiratory CO_2 were made in carbonate free NaOH.

[6] Armstrong, W. D., Schubert, J., and Lindenbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 233.

All samples, with the 2 exceptions noted above, were converted to $BaCO_3$ and the activity estimated using an end-window G-M tube. The over-all counting error by the method in use at the time these experiments were carried out was ± 4-5%.

Results and Discussion. The experimental data is summarized in Table I. The results confirm prior findings,[1,4] that isotopic carbon administered as CO_2 appears predominantly in carbons 3 and 4 of the glucose units of liver glycogen. The distribution of isotope in the degradation fractions from blood is qualitatively similar to that found in the glycogen.

The present data must be regarded with certain reservations. The material from blood includes the blood glucose, lactate, and perhaps other related intermediates in trace amounts. The activity, as determined, might, therefore have been contributed mainly by trace substances of very high activity. In view of the fairly low levels of activity noted in the respiratory CO_2, this does not seem likely. The quantitative differences between the fractions from blood and liver glycogen cannot be evaluated with certainty at present.

From the practical point of view, since the method, as applied, yielded degradation fractions from blood that mirrored those from liver glycogen, it should prove a useful tool. Additional experiments are in progress using labeled compounds known to give an isotope distribution pattern in liver glycogen different from that found in CO_2 fixation.

Summary. Experiments are reported in which it was found that CaC'^4O_3 implanted intraperitoneally in rats produced a qualitatively similar isotope distribution pattern in the liver glycogen, and blood glucose and lac-

tate. This result suggests that in isotope feeding experiments, analysis of the blood glucose and lactate may yield information comparable to that obtained by the degradation of liver glycogen. Additional experiments to put this question to a more critical test are in progress.

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17222. Activation of Antifungal Extracts of Actinomycetes by Ultrafiltration Through Gradocol Membranes.

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In the course of the purification of extracts from broth cultures of two soil actinomycetes showing antifungal activity, it was observed that solutions which were passed through gradocol membranes often possessed greater activity than the original solutions. Striking results were obtained repeatedly and under varying conditions. Since there may be a wide application in studies of antibiotics, it seems well to report the original observations. The extent and nature of the phenomenon are under investigation.

Materials and methods. Microorganisms. Two distinct actinomycetes, Nos. 47205[1] and 48240, isolated from soil and found to be antagonistic to fungi pathogenic for man, were grown in liquid media dispensed in 250-ml amounts in 1000-ml Erlenmeyer flasks. Strain No. 47205 was grown in glycerol-yeast extract broth containing 0.15% agar for from 9 to 12 days at approximately 28°C, and No. 48240 in glucose-tryptone broth for from 12 to 17 days at the same temperature. The composition of the media varied from the original formulas[1] only in that distilled water replaced the tap water. Four pathogenic fungi, *Candida albicans* No. 4567, *Cryptococcus neoformans* No. 45215, *Trichophyton mentagrophytes (gypseum)* No. 45141, and *Trichophyton rubrum (purpureum)* No. 4507 were employed as test microorganisms.

Antifungal tests. The extracts were assayed by the agar-diffusion or cup test and by agar-streak or agar-dilution tests.[2] Glucose-tryptone agar[1] was used throughout. The time of incubation varied according to the rate of growth of the particular test microorganism and the type of test. Thus in the diffusion tests in which *C. albicans* was employed, incubation for 8 to 10 hours was adequate, while with *C. neoformans*, 22 to 24 hours were necessary. The agar-streak tests with all 4 test microorganisms were incubated 72 hours. All tests were incubated at approximately 28°C.

Ultrafiltration. Gradocol membranes were prepared from nitrocellulose in the form of Parlodion according to the method described by Bauer and Hughes.[3] The average pore diameter of the several membranes varied from 52 to 64 mμ.* The solutions were passed through the membranes under positive pressure. After filtration, the membranes were washed by passing through some of the pure solvent. They were then removed from the filter and extracted with a small volume of 95% ethyl alcohol. Whenever the pH of a solution was determined before ultrafiltration, it was in the acid range.

Experimental. Different types of solutions showing antifungal activity were subjected to

[1] Schatz, A., and Hazen, E. L., *Mycologia*, 1948, **50**, 461.

[2] Reilly, H. C., Schatz, A., and Waksman, S. A., *J. Bact.*, 1945, **49**, 585.

[3] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol,*. 1934, **18**, 143.

* In one experiment with a coarse membrane, 378 mμ, ultrafiltration did not result in activation of the solution.