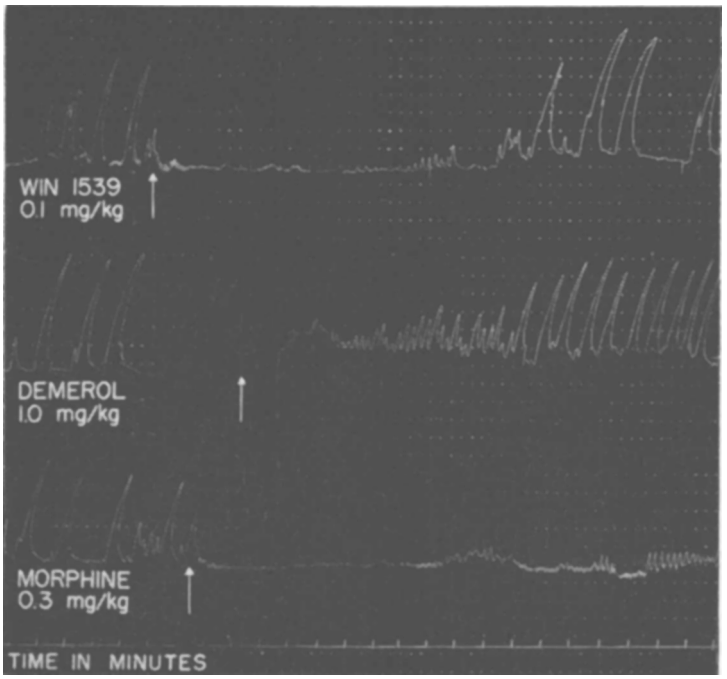

FIG. 2.
Kymographic recordings of intact stomach of anesthetized rat.

but morphine retarded emptying more than either of these drugs. Demerol caused a stimulation of the intact rat's stomach whereas WIN 1539 and morphine decreased the motility of this organ.

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17172. Some Relationships of Folic Acid to Structurally Similar Metabolites.*

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Jacobson and Good[1] observed that if folic acid (pteroylglutamic acid) is incubated with milk xanthopterin oxidase a material producing a greater response in cases of pernicious anemia than folic acid itself was obtained. At nearly the same time Kalckar and Klenow[2] demonstrated that xanthopterin is converted to leucopterin by milk xanthopterin oxidase and that this reaction is strongly inhibited by

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1 Jacobson, W., and Good, P. M., International Physiol. Congress, *Abstracts*, 1947, July.

2 Kalckar, H. M., and Klenow, H., *J. Biol. Chem.*, 1948, **172**, 349.

approximately equimolar amounts of folic acid. However, these workers observed that if folic acid is first incubated alone with the enzyme preparation the characteristic inhibition of xanthopterin oxidation is not observed.[3] They also showed later that this inhibitory effect is given to a much less extent by highly purified folic acid.[4]

Since the structure of the pterin nucleus is very similar to certain of the purines and also to the flavin prosthetic groups, it is possible that inhibition of enzymic reactions by folic acid is competitive in nature. Because of the same structural similarities the possibility also exists that folic acid can be metabolized to some extent by the same reactions. We have studied this problem in relation to both of these possibilities and have concluded that inhibition of purine metabolism *in vitro* by aldehyde-free folic acid does occur. Our results also indicate that folic acid itself may be metabolized to some extent by the same reactions.

Experimental. In order to observe the relationship between the *in vitro* metabolism of guanine, the purine most similar in structure to the pterin nucleus and folic acid, oxidation was followed using rat liver hemogenates prepared according to a method of Axelrod and Elvehjem.[5] Livers were obtained from adult, male albino rats maintained on stock ration for several months. The oxidation of guanine, folic acid and guanine plus folic acid was observed for 160 minutes using a conventional Warburg apparatus at 30°C. To each flask was added 1 ml of 16.6% liver homogenate. The levels of substrates employed were as follows: guanine, 0.2 ml 0.02 M; folic acid, 0.2 ml 0.02 M. In addition, 0.2 ml 1 x 10^{-4} M cytochrome-*c* and 0.2 ml water were added to each flask. When both guanine and folic acid were included in the same flask, the water was omitted. To absorb carbon dioxide, 0.2 ml 10% KOH was included in the center well of each Warburg vessel.

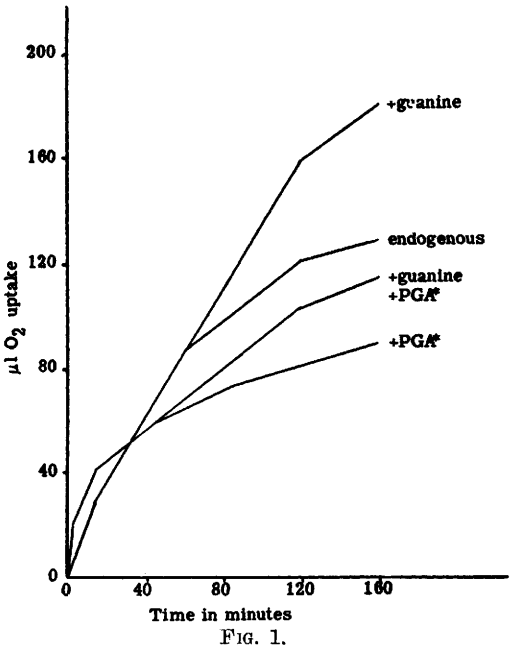

FIG. 1.

Oxygen uptake data showing the influence of folic acid upon guanine metabolism in rat liver homogenate.

* Aldehyde-free. See text.

Since oxidation of xanthopterin by milk xanthopterin oxidase has been shown to be strongly inhibited by samples of folic acid, a purified xanthine oxidase preparation diluted 5-fold with 0.039 M sodium potassium phosphate buffer (pH 7.5) was incubated with xanthine and xanthine plus folic acid (0.2 ml 0.02 M of each substrate). Oxygen uptakes were followed until oxidation of xanthine ceased.

Folic acid concentrations were made equivalent to concentrations of other substrates in respective experiments in order to observe relative oxidation of the substrates. Both commercial folic acid (folvite) and an aldehyde-free preparation of folic acid were employed separately to determine if aldehyde impurities are necessary for the inhibition of xanthine oxidase by folic acid. All substrates were made up in equivalent quantities of alkali.

Results. As shown by the curves in Fig. 1, folic acid strongly inhibits endogenous respiration in rat liver *in vitro*. Also metabolism of guanine (presumably via guanase to xan-

[3] Kalckar, H. M., and Klenow, H., *J. Biol. Chem.*, 1948, **172**, 351.

[4] Kalckar, H. M., Kjeldgaard, N. O., and Klenow, H., *J. Biol. Chem.*, 1948, **174**, 771.

[5] Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, **140**, 725.

thine and subsequent oxidation of the xanthine) is decreased about 50%. In the experiment shown aldehyde-free folic acid was employed although almost exactly similar results were obtained when commercial folic acid was employed. Because of the structural similarities between guanine and folic acid it appeared possible that the pterin nucleus could be metabolized similarly to guanine, *i.e.*, a hydrolysis and subsequent oxidation. However, such an effect as this involving oxidation would be obscured by the depression of endogenous respiration by folic acid. It is interesting to note that in the early portion of the curve for flasks containing folic acid there is a brief stimulation of oxygen uptake.

The similarity of the pterin nucleus to the flavin prosthetic groups and purines may possibly explain the depression of endogenous respiration and oxidation of the xanthine arising from guanine. It is also possible that guanase itself is inhibited competitively by folic acid.

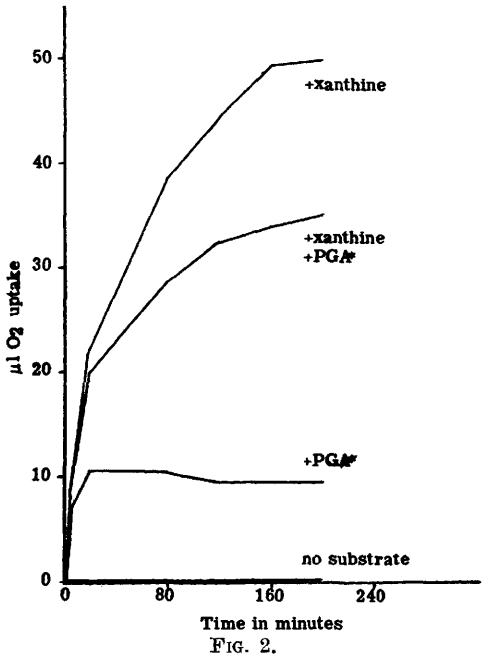

FIG. 2.

Oxygen uptake data showing the interrelationships of xanthine and folic acid in a purified xanthine oxidase preparation.

* Aldehyde-free. See text.

In Fig. 2 a typical experiment is presented in which a purified xanthine oxidase preparation was employed. Here oxidation of xanthine proceeds easily if it is present alone with the enzyme. However, this oxidation is strongly inhibited by folic acid. Some oxidation of folic acid appears to occur when it is included in the system alone with the enzyme. This oxidation, however, occurs very quickly and approaches a maximum in about one-half hour. This oxygen uptake might conceivably have arisen from oxidation of aldehydic impurities in the folic acid. However, in this experiment aldehyde-free folic acid was employed. Therefore, this small amount of oxidation appears to arise from oxidation of the folic acid itself, although the reaction is soon inhibited by the product or products formed. Whether the product of the reaction between folic acid and xanthine oxidase is identical with the "activated" folic acid of Jacobson is open to question at this time.

Summary. Folic acid whether commercial or aldehyde-free inhibits guanine metabolism and endogenous respiration in rat liver homogenates. Oxidation of folic acid itself in such systems is not clearly evident.

Oxidation of xanthine by a purified preparation of xanthine oxidase is strongly inhibited both by commercial and aldehyde-free folic acid. In a system containing only folic acid and the enzyme some oxidation of the folic acid appears to occur although this reaction is transient.

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