majority survived. Also, virus titers must be regarded as rough approximations. The organs of 12 to 30 animals were included in a pool. Therefore, the titer of the pool would be no more than approximately 1 log less than that of the single specimen with the highest value.

That the mouse infected with Coxsackie virus during the first 2 days of life produces little or no interferon by the third day after infection does not necessarily mean that its cells are incapable of elaborating this substance, given sufficient time. It was not possible to test beyond the third day since most of the animals were dead by the fourth day. (When inocula greater than 100 LD_{50} were used suckling mice died before the third day.) Nonetheless, from our data as well as from those of Isaacs and Baron(10) for the chick embryo and those of Sawicki(11) for Sendai virus infected mice, it would appear that the newborn animal does differ markedly from the older one in the way in which interferon is released in response to infection. Whether or not this is significant in explaining the severity of certain infections in the young is yet to be determined. However, available evidence would support the hypothesis that the failure to produce interferon is one of the important handicaps under which the young individual suffers in coping with virus infections.

Summary. Mice 24 hours to 6 weeks old were infected with Coxsackie B1 virus. Their tissues were assayed for virus and interferon content. It was demonstrated that adult mice produced interferon in all the tissues which became infected, whereas suckling mice produced small amounts of interferon in their livers only. There is a direct relationship between interferon titers and rapidity with which virus disappears from the tissues. A hypothesis is offered that the difference in outcome of Coxsackie B1 infection in the suckling and older mouse is in large part to be explained by inability of the cells of the immature animal to elaborate interferon.

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Received December 30, 1963. P.S.E.B.M., 1964, v115.

Folate Activity in Tissues of Rats on Folate-Free Diets.* (29087)

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Recently we presented data on folate activity in blood and various tissues in rats kept on a standard diet(1). No detailed information is available on the effect of folate deficiency on the folate tissue stores, nor is it known to what extent blood folate activity reflects the state of the body folate stores. Increased urinary excretion of formiminoglutamic acid was found in folate-deficient rats after 20-40 days on a folate-free diet(2).

* This work was supported by grants from the B. de Rothschild Foundation for Advancement of Science in Israel, Inc., and Rebecca Rose Memorial Fund, New York.

The present communication deals with the effect of folate-free and pteroyl-glutamate (PGA) supplemented diets on the blood, kidneys and liver folate activity in the rat.

Material and methods. Weanling rats, weighing from 30-50 g, of the Hebrew University Animal House, were used; each rat was kept in a separate metabolic cage to prevent coprophagy. The animals were divided into 3 equal groups according to the diet given; Group 1 was fed a folate-free diet similar to that employed by Rabinowitz and Tabor(2) consisting of (g/kg) of: cornflour —730, casein (vitamin-free)—180, vegetable oil—50, salt mixture—40, and various vitamins (mg/kg): biotin—1, choline chloride—2,000, calcium pantothenate—20, inositol—33, menadione—20, niacin—50, p-aminobenzoic acid—40, pyridoxine—2.65, riboflavin—3.3 and thiamin—3.3. Vit. A (200 units) and Vit. D (20 units) were given orally, twice weekly by pipette. In another group of animals Vit. B_{12} (30 μg/kg diet) was added to the diet (Group IA). Group II rats received the folate-free and Vit. B_{12}-free diet, supplemented with PGA (20 mg/kg diet). In some experiments a much smaller amount of PGA was added (4 mg/kg diet; Group IIA). Group III rats were kept on a standard mixed diet with a folate activity of 0.5 mg/kg (assayed with *L. casei*). The composition of the standard diet was the following (g/kg): whole corn meal—670, alfalfa meal—50, soya bean meal (protein content—44%)—160, fish meal—40, calcium carbonate—60, dicalciumphosphate—15, sodium chloride—4, and vitamin mixture containing also Vit. A and D—2.5. Sulfaguanidine (2%) was included in all the diets used. A total of 180 rats were employed in these experiments.

After 10 and 20 days on the different diets, the animals of each group were sacrificed by bleeding. The blood, livers and kidneys of 3 rats were pooled and assayed. The blood was collected over heparin, and hemoglobin concentration was determined (cyanmethemoglobin). Folate activity was estimated in whole blood using *L. casei* and *P. cerevisiae* assays (3). The liver and kidneys were removed from the animals, rinsed in physiological saline, blotted on filter paper, weighed and homogenized as described previously(1). Samples of tissues were assayed for folate activity immediately after homogenization as well as after autolysis, *i.e.,* after incubation in the presence of ascorbate for 18 hr at 37°C under toluene(1). The purpose of autolysis was to allow for transformation of folates that were active only for *L. casei* (*e.g.,* N-5-methyl-tetrahydrofolate) into forms measurable also with *P. cerevisiae*(4,5).

Results. Folate activity determinations of various tissues have shown that the bulk of activity was present in the liver and in the kidneys. Blood was also found to contain considerable folate activity(1). Changes in the folates of these tissues in rats fed folate-free and PGA-supplemented diets are reported below.

Kidneys. A marked drop in folate activity was observed in the kidneys of rats kept for 10 days on the folate-free diet. With both assays, the drop amounted to about 70% as compared with that found in kidneys of rats fed the standard diet. After 10 additional days on the folate-free diet, folate activity as measured with the *L. casei* assay dropped to 13% of the control rats, and 20% was recovered with the *P. cerevisiae* assay. No significant differences were noted in folate activity, regardless of whether the tissues were or were not autolyzed prior to the bioassay (Fig. 1). A similar, although a slightly lesser, drop in folate activity was seen in rats which received the same diet but supplemented with Vit. B_{12} (Table I). No increased folate activity was found in the kidneys of

TABLE I. Folate Activity in Liver and Kidneys of Weanling Rats After 10 Days on Various Diets.*

Diet used	Liver (μg/g tissue)	Kidneys (μg/g tissue)
Standard	6.0	3.0
Synthetic + PGA (20 mg/kg)	16.2	3.2
Idem + B_{12} (30 μg/kg)	14.4	3.9
Synthetic + PGA (4 mg/kg) + B_{12} (30 μg/kg)	9.8	2.55
Synthetic + B_{12} (30 μg/kg)	4.7	1.7
Synthetic (Vit B_{12} & PGA omitted)	2.4	1.2

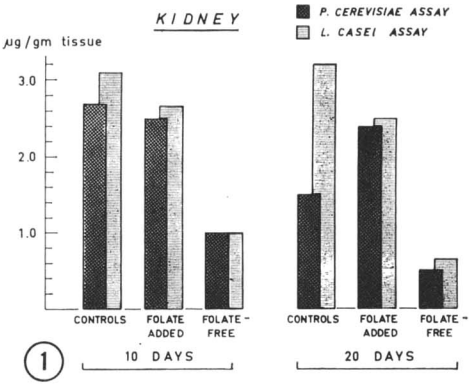
* Folate activity was determined with *L. casei* on autolyzed samples only. For details see *Methods.*

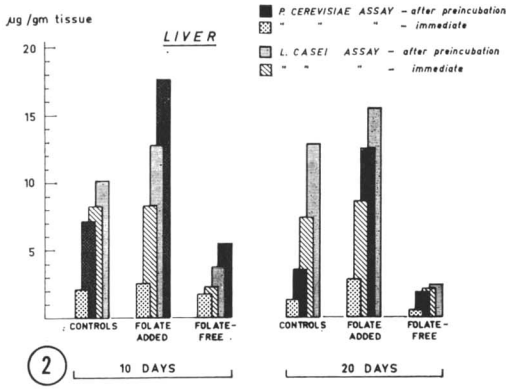
rats fed a diet supplemented with either 4 or 20 mg/kg of PGA (Fig. 1, Table I).

Liver. a) After autolysis: A 40% drop in liver *L. casei* folate activity was seen in the animals kept on a folate-free Vitamin B_{12}-free diet for 10 days, as compared to rats fed the standard diet. However, the drop was not significant when activity was measured with *P. cerevisiae*. After an additional 10 days on the folate-free Vitamin B_{12}-free diet the *L. casei* activity of the liver dropped to 20% of that found in the control rats, whereas the *P. cerevisiae* activity decreased by 50%. The animals with PGA added to the diet (either 4 or 20 mg/kg) showed a folate activity almost twice as high as those kept on the standard diet (Fig. 2, Table I). In these rats the activity as measured with *P. cerevisiae* exceeded the one measured with *L. casei* (Fig. 2).

b) Without autolysis: Bioassays performed on rat liver homogenates without prior autolysis showed much lower activity than the same tissues after autolysis. This was particularly true with regard to livers of animals fed supplementary PGA and those fed the standard diet. The difference between the liver *L. casei* activity with and without autolysis in animals on a folate-free diet was insignificant.

Whole blood. Hemoglobin values in folate-deficient and control rats were similar and ranged from 10.4 to 12.3 g%. The blood folate activity of rats on a folate-free Vitamin B_{12}-free diet showed no significant changes after 10 days; however, 20 days after the beginning of the experiment a 70% drop in blood folate activity was noted with both *L. casei* and *P. cerevisiae* assays. The blood *L. casei* and *P. cerevisiae* activities in animals on diets supplemented with PGA (20 mg/kg) were much higher than that of control rats fed a standard diet (Fig. 3).





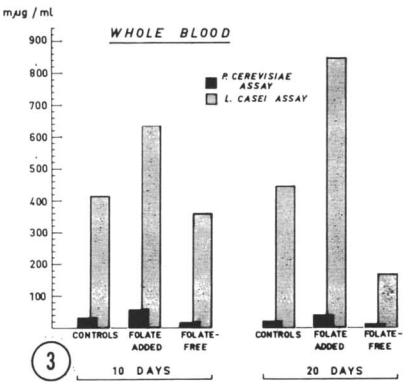


FIG. 1. Folate activity in rat kidney obtained from animals on standard (controls), folate-free and PGA-supplemented diets, respectively. Note marked decrease in folate activity after 10 days on folate-free diet. Each value given is the calculated average based on 36 rats.

FIG. 2. Folate activity in rat liver from animals on standard (controls), folate-free and PGA-supplemented diets, respectively. Average folate activities are given both in liver tissue assayed immediately after homogenization and after 18 hr incubation at 37°C of the sample (autolysis) prior to assay. Note marked increase in folate activities following autolysis in animals fed the standard diet or a synthetic diet supplemented with PGA. No such increase could be found in livers of animals on a folate-free diet. Values given represent averages of 36 animals in each group.

FIG. 3. Whole blood folate activities in rats kept on a folate-free, PGA-supplemented and a standard (controls) diet for 10 and 20 days, respectively. Values given are averages of 36 animals.

Discussion. These data show a rapid depletion of folates in liver and kidneys of rats rendered deficient by elimination of PGA and Vitamin B_{12} or PGA alone from their diet. After 20 days on a folate-free diet only about 10-20% of the original folate activity could be recovered in these organs. It was also observed that folates stored in the liver and kidneys decreased considerably after 10 days before there was a significant drop in folate activity of the blood. It can be concluded, therefore, that in the rat decreased blood folate activity indicates a severe depletion of tissue stores. As no anemia developed in any of the animals examined, it follows that a diminished blood folate activity precedes the development of anemia.

In another series of experiments in which the animals were deprived of folates and not of Vit. B_{12}, a similar, though slightly lesser, drop in folate activity was encountered (Table I).

Another aspect of folate metabolism has to be discussed in the light of the data presented here. Donaldson and Keresztesy(5) reported that N-5-methyl-tetrahydrofolate constitutes the main form of folates in horse liver, and exhibits growth promoting activity towards *L. casei* but not to *P. cerevisiae.* Herbert *et al*(6) arrived at a similar conclusion regarding the folates in human serum. The present study reveals that a considerable part of the folate activity of unautolyzed rat liver may be due to the presence of N-5-methyl-tetrahydrofolate, since it was measurable with *L. casei* and not with *P. cerevisiae.* Autolysis increased considerably the folate activity measurable with *P. cerevisiae* in the liver. This increase has to be ascribed to the transformation of methyl-tetrahydrofolate into forms active to *P. cerevisiae* (*e.g.,* hydroxymethyl tetrahydrofolate, tetrahydrofolate and other forms) in accordance with the findings of Donaldson and Keresztesy(5) and other authors. On the other hand, after autolysis the activity as measured with *L. casei* also increased and was often much higher than before autolysis. The latter finding could be explained by the assumption that during autolysis some unknown form(s) of folates (*e.g.,* polyglutamates) were released or degraded and became available to *L. casei.*

The storage function of the liver for folates is well demonstrated by the finding that addition of PGA to the diet resulted in a substantial increase of the liver folate activity but not of that in the kidneys.

Summary. Rats kept on a folate-free diet were found to have decreased folate activity in kidneys, livers and blood in comparison with control animals kept on a mixed standard diet. In the kidneys the drop in folate activity amounted to 70% after 10 days, and to about 85% after 20 days. No appreciable differences were encountered, regardless of whether or not the kidneys were autolyzed prior to the bioassay, or whether the bioassay was performed with *L. casei* or *P. cerevisiae.* A 40% drop in folate activity was found in livers of animals kept on a folate-free diet for 10 days; the drop amounted to 80% after 20 days. These results were obtained when autolyzed tissues were employed in the bioassay. Livers assayed without prior autolysis showed very low activity with *P. cerevisiae* because of the high contents of methyl-tetrahydrofolate of the liver. After autolysis similar activities were obtained with both assays. Decreased blood folate activity was found in rats only after they had been kept for 20 days on the deficient diet. Thus, decreased folate activity in the blood indicates a severe depletion of the tissue stores. Rats kept on a synthetic diet supplemented with PGA (20 or 4 mg/kg diet) showed a higher liver and blood folate activity than control rats fed on mixed standard diet. The livers of both groups showed almost double folate activity when assayed on autolyzed samples than when done without such treatment. Autolysis presumably made available to *L. casei* forms of folates (*e.g.,* polyglutamates) to which it could not respond prior to the treatment.

The valuable technical assistance of Mrs. M. Jablonska is gratefully acknowledged.

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Received December 31, 1963. P.S.E.B.M., 1964, v115.

Stability of Bacterial Flora with Long-Term Iodochlorhydroxyquin Therapy. (29088)

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A successful therapeutic and prophylactic control program, with the use of iodochlorhydroxyquin, for *Entamoeba histolytica* and *Shigella sonnei* dysenteries has been reported (1). The initial group of nearly 1300 patients has been expanded to approximately 4000 patients(2). Although long-term administration of iodochlorhydroxyquin has not resulted in any clinical manifestation of adverse bacteriological flora alteration, nor in any fungal disorder, it seemed advisable to confirm these clinical impressions with detailed bacteriological studies. Accordingly, a double-blind study was designed to investigate the possible bacterial and mycotic flora changes in the nasopharynx and intestinal tracts of subjects treated with iodochlorhydroxyquin.

Method. Twelve children, inmates of the Sonoma State Hospital, were chosen at random from the hospital population to be subjects for this study. Six were mongoloids (Down's Syndrome), and 6 had other diagnoses for the etiology of their mental retardation. All subjects were free from medical diseases other than the reason for hospitalization.

Drug and placebo therapy were administered in code by the trained nursing staff without any knowledge of what the medications were. Three mongols and 3 nonmongols, selected at random from the subject group, were given iodochylorhydroxyquin (Entero-Vioform®),* 250 mg three times daily with meals. The other 6 subjects (3 mongols and 3 nonmongols), also selected at random, were given placebo medication.

Specimens for bacteriological examination taken by swab from nasopharynx, throat, and rectum were submitted by code to an independent laboratory† not associated with the State Hospital. The microbiologist was unaware of which subjects received iodochlorhydroxyquin and which ones received placebo therapy. Control bacteriological examinations were performed before therapy on 2 occasions. Specimens were then taken at the end of 1, 2, 4, 8, and 12 weeks of continuous therapy.

Each nose, throat, and rectal swab was inserted into Stuart's transport medium and sent to the laboratory within 24 hours. Upon arrival, various types of culture media, which are widely employed for bacterial growth and identification, were inoculated from these swabs. Incubation was carried out overnight at 37° temperature.

After the incubation period, all growths were examined macro- and microscopically. Microorganisms present were subcultured by "pure" colony pick into broth. Confirmation of "purity" was established by Gram stain smear. If "pure," these subcultures served as inoculi for bacterial identification proce-

* Furnished by Ciba Pharmaceutical Company, Summit, N. J., through the kindness of Mr. Eliot L. Poston.

† Foundation for Clinical Pharmacology, Inc., Berkeley, Calif.