water contents of rat atria stimulated for 3 hours in Krebs-Ringer bicarbonate medium containing 6 mM potassium were not significantly different from those of freshly dissected atria, indicating that the medium used in the present investigation is physiological with respect to its potassium concentration and osmotic pressure. Atria stimulated for 2 hours in K^+-free medium, after a one hour equilibration period in the normal medium, showed a 57.4% loss in potassium content. No significant change in water content, however, was observed under these conditions. Atria stimulated 200 times per minute in K^+-free medium showed idiopathic rhythmicity, contracture, and a temporary increase in contractile force. Self-stimulated atria placed in K^+-free medium showed a temporary period of increased rate which was sensitive to the chronotropic action of epinephrine. This was followed by a phase of very slow rate and of epinephrine insensitiveness. Arrhythmias were observed in both phases. The degree of contracture observed was directly related to the rate while the force of contraction was inversely related. The intrinsic rate showed a direct relationship to Ca^{++} concentration both in presence and absence of potassium.

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Folate Activity in Rat Tissue Before and After Pteroylglutamic Acid Load.* (28084)

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Human blood has been found to contain a variety of folic acid-active substances(1,2). With simultaneous use of 3 different bio-assays (*Lactobacillus casei, Streptococcus faecalis* and *Pediococcus cerevisiae*) it has also been established that the ratio of the activities of *P. cerevisiae:S. faecalis:L. casei* in normal human blood and serum is 1:2:15. In moderate anemias due to folic acid deficiency, the *L. casei* values were the first to decrease (2), whereas in severe nutritional megaloblastic anemia all 3 bio-assays revealed extremely low activity both in whole blood and in serum(3). It seemed of interest to learn whether the distribution of the various folate derivatives in the major storage organs, such as the liver, is similar to that found in the circulating blood and, furthermore, to what extent this distribution may be affected by loading with pteroylglutamic acid (PGA).

The present paper deals with the results of such an investigation in the rat.

Material and methods. Seventy random-bred female white rats, weighing between 250-300 g, were used. Three animals were kept in a cage and were fed a standard, mixed diet. Food was withheld for 24 hr before, and for an additional 48 hr after the load. Thirty-eight rats received pteroylglutamic acid (PGA) 2 μg/g body weight, by intra-

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TABLE I. Folate Activity in Various Rat Tissues Before and at Intervals After Loading with PGA.*

	Before load	Hr after injection of PGA				
		1	3	6	24	72
			Blood			
L. casei	.2	.8	.3	.3	.2	.2
S. faecalis	.01	.6	.1	.04	.06	.05
P. cerevisiae	.008	.009	.01	.008	.007	.01
			Liver			
L. casei	9.4	9.8	8.6	11.5	8.2	8.1
S. faecalis	5.5	7.8	7.6	7.3	7.0	6.5
P. cerevisiae	5.5	7.5	—	—	5.6	6.0
			Kidney			
L. casei	1.6	4.8	1.8	1.9	2.3	2.1
S. faecalis	1.4	5.1	2.6	2.2	2.3	1.7
P. cerevisiae	1.4	1.8	1.8	1.7	1.8	1.8
			Spleen			
L. casei	.6	1.7	.7	.8	1.1	.8
S. faecalis	.3	1.2	.3	.4	.7	.7
P. cerevisiae	.3	.2	.3	.3	.3	.3
			Intestine			
L. casei	.6	1.4	1.0	1.1	.7	.5
S. faecalis	.3	1.2	.5	.4	.4	.4
P. cerevisiae	.2	.3	.3	.2	.3	.3

* All values are expressed in μg/g tissue. PGA was used as standard for both *L. casei* and *S. faecalis*, while folinic acid (Leucovorin)—for *P. cerevisiae*; the results are expressed in terms of PGA and folinic acid, respectively. The assay medium contains ascorbic acid. Assay procedure is described elsewhere(4).

peritoneal injection. At intervals of 1, 3, 6, 24 and 72 hr after injection of PGA (or of saline in the control animals), groups of 3 rats were sacrificed by bleeding through cardiac puncture. The blood was drawn into heparinized syringes, frozen immediately, and stored at –18°C until tested. Liver, spleen, kidneys, small intestines and the stomach were rinsed in saline, blotted with filter paper, weighed and stored at –18°C until tested.

Blood samples for assay of folate activity were prepared as described elsewhere(4). The various tissues were homogenized in the cold with 5 ml of 0.25 M phosphate buffer, pH 7.0. Ascorbic acid (5 mg/g tissue) was added and incubated for 18 hr at 37°C under toluene. Following incubation the pH was lowered to 6.0 with dilute HCl, and the samples were autoclaved to precipitate the proteins; the supernatants were used for folic acid determinations. The blood and tissues were examined microbiologically by 3 assays: *L. casei* ATCC No. 7469 was used to determine "total folate activity" as it responds to the conjugated and free forms of folic acid, to the tetrahydrofolic acid group, and also to N^5-methyl-tetrahydrofolic acid(5,6). *S. faecalis* No. 9 was employed to measure free folic acid and its reduced forms, and *P. cerevisiae* ATCC No. 8081 served to test the activity of folinic acid and related reduced compounds. The assay methods are described elsewhere(4).

Results. The average folate activities of the various tissues as measured by the 3 bioassays were as follows (Table I).

Liver. The liver showed the highest activity, 9.4 μg/g tissue (wet weight) with the *L. casei* assay. The activities with each of the other 2 micro-organisms were lower and amounted to 5.5 μg/g, respectively. The injection of PGA was followed by a slight increase in activity with the *S. faecalis* assay which persisted for 24 hr (Table I).

Kidneys. Pre-injection values obtained in the kidneys were 1.6 μg/g tissue for *L. casei* and 1.4 μg/g for *S. faecalis* and *P. cerevisiae*, respectively. A 3-4-fold increase in activity for *L. casei* and *S. faecalis* followed the injection of PGA. Three hours after the injection the values found with the *L. casei* assay returned to the preinjection level, while they

remained slightly elevated for 24 hr when measured by *S. faecalis*. A slight increase was seen in the *P. cerevisiae* values following the injection, which persisted throughout the observation period of 3 days (Table I).

Spleen. The *L. casei* assay revealed an activity of 0.57 μg/g tissue. The activities found with *S. faecalis* and *P. cerevisiae* were 0.34 and 0.16 μg/g, respectively. Injection of PGA caused a 2-4-fold increase in the activity as measured by *L. casei* and *S. faecalis*, while no such increase was noted in the *P. cerevisiae* assay. The increased values persisted in some animals for up to 24 hr (Table I).

Intestine. The folate activity of the stomach and intestines was found to be 0.6 μg/g with *L. casei*, 0.33 μg/g with *S. faecalis* and 0.10 μg/g with *P. cerevisiae*. Following injection of PGA a 2-4-fold increase was observed in the *L. casei* and *S. faecalis* values, which lasted up to 6 hr. A slight but persistent increase was also found in the activity as measured by the *P. cerevisiae* assay (Table I).

Blood. Marked differences were noted in the blood folate activity depending on the assay employed. With *L. casei* a value of 0.20 μg/ml was obtained,† whereas with *S. faecalis* the activity was as low as 0.01 μg/ml, and the value obtained with the *P. cerevisiae* assay was still lower, 0.008 μg/ml. The activities of *P. cerevisiae, S. faecalis* and *L. casei* assays were in the ratio of 1:1.3:25. One hour after the injection a 3-fold increase was observed with the *L. casei* assay, a 60-fold increase with *S. faecalis*, while no elevation was found with the *P. cerevisiae* assay. Two hr later the *L. casei* values had returned to the pre-injection level, while the *S. faecalis* activity was still about 10 times higher than control levels, and remained elevated throughout the period of observation (Table I, Fig. 1).

Comment. Folate activity of normal human and animal tissues has been determined by several investigators. Mitchell and Isbell

† In another group of younger animals, not included in this series (average weight 110 g), higher values were found in whole blood with the *L. casei* assay.

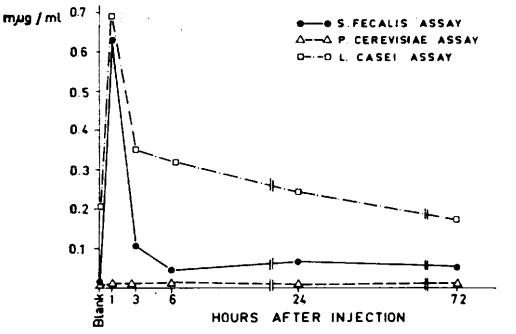

FIG. 1. Folate activity of whole blood before and at intervals after intraperitoneal injection of folic acid (PGA, 2 μg/g body wt). Note marked post-load increase in activity, particularly as measured with *S. faecalis*, as against the lack of appreciable change on values of the *P. cerevisiae* assay. Values represent averages of 7-8 animals for each point.

(7), with the aid of the *S. lactis* R. assay, found the following values in rat tissues: liver–5.9 μg/g, kidney–8.8 μg/g and spleen–5.8 μg/g tissue. These authors used a folic acid concentrate of a "potency of 40,000" as standard. With the same assay Pitney and Onesti(8) found between 0.6-1.6 μg/g activity in normal human liver tissue. With *S. faecalis*, Mulgaonkar and Sreenivasan(9) found the folate activity in rat liver to be 6.3 μg/g tissue. With *P. cerevisiae*, Noronha and Screenivasan(10) found an activity of 7.5 μg/g rat liver tissue. Other investigators found widely varying activities in human and rat tissues with *S. faecalis* and *P. cerevisiae* (11-18). Toennies *et al.*(14), using *L. casei* as the test organism, found in rat whole blood an activity of about 0.2 μg/g.

The high *L. casei* activity in whole blood might be due either to polyglutamates or to a special form of tetrahydrofolic acid (*e.g.*, N^5-methyl-tetrahydrofolate) to which *S. faecalis* and *P. cerevisiae* respond feebly or not at all. In rat tissues, such as liver, spleen, kidney and small intestine, 50% or more of the folates are present as various tetrahydrofolate derivatives (measurable by *P. cerevisiae*). In experiments not included here, in which the folate assays were performed immediately after the animals had been sacrificed, *P. cerevisiae* values were as high as those obtained with *L. casei*(19).

These findings are in apparent contradiction to those reported by Donaldson and

Keresztesy(17,18) who showed that most of the folate is present in horse liver as methyl-tetrahydrofolate, and thus inactive for *P. cerevisiae*. This discrepancy can be explained by the fact that these authors measured the folate activity without prior autolysis, whereas in our case the determinations were performed after incubating the tissue extracts for 18 hr at 37°C under toluene in presence of ascorbate (see *Methods*). The prolonged incubation allowed the transformation of methyl-tetrahydrofolic acid into forms to which both *S. faecalis* and *P. cerevisiae* respond. The presence in the liver of enzymes performing these transformations has been demonstrated(17). Indeed, assays performed in liver tissue without prior autolysis(19) yielded results similar to those observed by Donaldson and Keresztesy(17,18) and Silverman *et al.*(16).

Following PGA injection in rats, similar increases in activity were found both with *S. faecalis* and *L. casei*, whereas none or very little increase was found with the *P. cerevisiae*. Thus, it is reasonable to conclude that in whole blood as well as in other organs the higher *L. casei* activity resulted from the increase in PGA. The lack of significant increase in the values obtained with the *P. cerevisiae* assay in any of the organs examined may be explained by the relative slowness of the metabolic transformations of PGA into one of its reduced forms. Similar conclusions for humans were drawn by Johns *et al.*, using tritiated folic acid(20).

Summary. Folate activity was determined in the whole blood and tissues of 70 normal rats, before and after loading with PGA using 3 different bio-assays simultaneously. *L. casei* was used to measure "total folate activity," *S. faecalis* to estimate monoglutamate activity as well as that of the reduced forms of PGA, and *P. cerevisiae* to determine only the reduced folate activity.

L. casei activity before loading with PGA was about 25 times higher in the peripheral blood than when measured with *S. faecalis* and *P. cerevisiae*. On the other hand, the ratio of activity with *L. casei, S. faecalis* and *P. cerevisiae* in liver, kidney, spleen and intestines was only 1.4:1:1. It was concluded that the reduced forms constitute over 50% of the folate derivatives in rat tissues.

Intraperitoneal injection of 2 μg/g PGA was followed by a temporary elevation of the activity with the *L. casei* and *S. faecalis* assays which disappeared within 6-24 hr after the injection. No significant increase was noted in the *P. cerevisiae* assay following PGA load.

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