B_{12} and the desoxyribosides of guanine and hypoxanthine. These observations are in full accord with direct growth experiments (Table I).[10] *L. leichmannii* showed no zone of growth on the chromatogram in the position where the "citrovorum factor" was located. Thus the data of Table III provide additional evidence for the separate identities of the factors required by *L. leichmannii* and *L. citrovorum* and demonstrate the existence in liver of an unknown component, not identical with thymidine, that promotes the growth of *L. citrovorum*.

Liver extract, treated with alkali as described in Table I gave results in a paper strip chromatogram identical with those shown in Table III except that vitamin B_{12} was destroyed as indicated by lack of growth of *L. leichmannii* in the region appropriate to vitamin B_{12}.

Summary. 1. *Leuconostoc citrovorum* was found to respond to a growth-promoting factor in the concentrated liver extract, but this organism did not respond to vitamin B_{12}. The alkali-stable nature of the "citrovorum factor" further contrasts it with vitamin B_{12}. 2. Two fractions were separated from liver extract by paper strip chromatography; one of these fractions was presumably thymidine and promoted growth of *Lactobacillus leichmannii* and *Leuconostoc citrovorum*. The other fraction was inactive for *L. leichmannii* but active for *L. citrovorum*.

3. Although thymidine or high levels of pteroylglutamic acid (PGA) when tested singly were only partially effective in promoting growth of *L. citrovorum*, the simultaneous addition of thymidine plus PGA produced marked growth of the organism. This finding suggests a functional relationship between thymidine, PGA and the "citrovorum factor."

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17253. The Lipotropic Effect of Estrogenic Hormones in Inbred Rats.*

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It has been shown[1,2] that estrogenic hormones exert in rats distinct lipotropic activity. In particular, estrogenic compounds (estrone, estradiol benzoate, ethinyl estradiol) allow a more efficient use of methionine as a lipotropic agent. In these studies, various possible sources of error were taken in consideration, such as difference in food intake, weight, sex and genetic identity. Further, it has been found necessary to run control and treated rats simultaneously in all experiments. This latter precautionary measure was prompted by the observation that experimental groups when not run simultaneously often differed in their absolute response even under identical experimental conditions.

With the exception of a few experiments in which unidentified strains obtained from a local dealer were employed, the studies were carried out on rats of the Sprague-Dawley strain.[1,2] In view of the observation that various inbred strains may vary more than 500% in their ability to inactivate estrogen[3] it became necessary to extend the observations on the lipotropic activity of estrogens to other strains beyond those used in previous studies,[1,2] especially to inbred strains, such as the Fischer strain, which shows "both a high threshold for vaginal estrus and an im-

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1 György, P., Rose, C. S., and Shipley, R. A., *Arch. Biochem.*, 1947, **12**, 125.

2 György, P., Rose, C. S., and Shipley, R. A., *Arch. Biochem.*, 1949, **22**, 108.

3 Segaloff, A., and Dunning, W. F., *Endocrinology*, 1946, **39**, 289.

paired ability for hepatic estrogenic inactivation".[3] In connection with the established lipotropic activity of estrogens in the strains hitherto tested[1,2] the claim that in the Fischer strain "extensive fatty infiltration of the liver develops when massive doses of estrogen are administered" (alpha-estradiol) appeared to be of particular interest. Thus, the possibility that the response of the inbred Fischer strain to estrogens might differ from that previously established in other strains, made it desirable to test the effect of estrogen on rats of this strain kept on an alipotropic diet.

Experimental. The same general procedures were followed as were previously reported.[1,2] Young adult rats, weighing 120-180 g, were used. All of the ahimals were bred and raised in our laboratory from rats of the Fischer strain received, through the courtesy of Dr. A. Segaloff, from Dr. W. F. Dunning (Wayne University, College of Medicine, Detroit). As usual, control and treated animals were run simultaneously in all experiments. When all of the animals of an experiment could not be run at one time, equal numbers of the various groups were started together. All of the animals received the same alipotropic diet which was of low protein-high fat type. It, and the vitamin supplements, were described in the previous paper.[1] In the experiments with ethinyl estradiol the hormone reduced the appetite of the rats to such an extent that it was necessary to run pair-fed controls.

As estrogens, estrone and ethinyl estradiol† were used, given orally,[2] the daily dose being dissolved in 2 drops of cottonseed oil. Control animals were given 2 drops of oil daily. When methionine was given, the daily dose (50 mg dl-methionine),‡ was dissolved in 1 ml of water, and mixed with the vitamin B supplement. In certain of the experiments, where a number of the animals did not drink this mixture, it was given to all by stomach tube.

At the end of the 21 day experimental period, the animals were sacrificed and the liver analyzed for total lipide with the same technique used in the previous experiments.

Results and Discussion. The results obtained are summarized in the attached Table. The most conspicuous difference in the reactivity of this inbred strain in comparison with that of other strains manifested itself in the very high fat content of the liver in the untreated animals fed the basal alipotropic diet. The average fat content of the liver both in male and in female controls of the inbred strain was found to be above 30% while with other strains in previous work[1,2] the corresponding figures were, as a rule, lower than 25%, and frequently less than 20%.

According to our observations made earlier, as well as to reports in the literature,[4] even within one experimental group the fat content of the liver shows often very considerable variations, with a distribution curve of wide extremes. In contrast, animals of the inbred Fischer strain of the present series showed remarkable uniformity of response. For instance, among 18 control animals of Experiments 21 and 22 (Table) only one rat showed a relatively low discrepant figure for liver fat, 14.6%, while the figures for all remaining 17 rats varied only between 30.8 and 43.8%, an exceptionally narrow range for this type of experiment.

Even with the use of the Fischer strain the lipotropic effect of estrogens could clearly be demonstrated, but only with ethinyl estradiol and not with the much weaker estrone, at least not with the doses of estrone given. Addition of methionine increased the lipotropic effect of ethinyl estradiol considerably but did not accentuate the effect of estrone. Even in genetically less homogeneous strains of rats estrone acted as a weak lipotropic agent or was found to be devoid of any lipotropic activity.[1,2] It is probable that in the highly inbred Fischer rats, kept on the basal alipotropic diet, the development of very intensive fat infiltration of the liver may exert a special inhibitory influence as to the manifestation of the lipotropic effect of estrogens. In further consequence, it is not surprising that

† Ethinyl estradiol was kindly supplied by Roche-Organan, Inc., Nutley, N. J.

‡ Kindly furnished by Wyeth, Inc.

[4] Beveridge, J. M. R., Lucas, C. C., and O'Grady, M. K., *J. Biol. Chem.*, 1945, **160**, 505.

TABLE I.
Liver Fat Experiments on Fischer Rats.

Exp. No.	Date	Group	No. of rats	Sex	Treatment (daily)	Liver		Rat weight		Food intake, g/day
						Wt (g)	Total fat (%)	Initial (g)	Change (%)	
21	10-17-47	a	8	M	—	10.6 ± 0.48	37.4 ± 1.5	164 (152-184)	—10.9 ± 1.3	7.7 ± 0.14
	to	b	9	M	Methionine (50 mg)	9.4 ± 0.79	28.3 ± 2.3	166 (150-182)	— 3.5 ± 1.3	7.6 ± 0.21
	11-18-47	c	9	M	Estrone (30 γ)	11.3 ± 0.55	38.6 ± 0.73	167 (153-182)	—11.8 ± 1.4	7.5 ± 0.21
		d	9	M	Methionine (50 mg) + Estrone (30 γ)	8.4 ± 0.39	22.3 ± 2.5	163 (153-180)	— 3.2 ± 1.5	7.1 ± 0.30
22	11-18-47	a	8	F	Methionine (50 mg)	6.9 ± 0.33	20.6 ± 2.4	158 (148-163)	— 3.6 ± 1.5	6.0 ± 0.13
	to	b	8	F	Methionine (50 mg) + Estrone (30 γ)	7.9 ± 0.69	23.5 ± 3.8	158 (147-170)	— 9.1 ± 1.2	5.4 ± 0.17
	11-29-47									
24	6-12-48	a	10	M	—	7.8 ± 0.40	33.1 ± 2.3	164 (135-196)	—21.0 ± 1.7	4.1 ± 0.24
	to	b	10	M	Ethinyl estradiol (30 γ)	8.0 ± 0.33	26.0 ± 1.3	165 (140-182)	—21.9 ± 1.4	4.2 ± 0.21
	6-25-48									
	8- 2-48	c	10	M	Methionine (50 mg)	6.0 ± 0.45	18.7 ± 2.9	149 (134-160)	—14.0 ± 1.3	4.2 ± 0.28
	to	d	10	M	Methionine (50 mg) + Eth. est. (30 γ)	6.2 ± 0.22	5.7 ± 0.11	152 (139-165)	—16.9 ± 1.4	4.6 ± 0.11
	8-27-48									
25	6-25-48	a	11	F	—	7.4 ± 1.3	31.5 ± 2.2	126 (121-152)	—21.2 ± 1.9	3.6 ± 0.34
	to	b	11	F	Eth. est. (30 γ)	7.4 ± 0.50	28.4 ± 1.7	132 (128-150)	—24.7 ± 2.1	3.7 ± 0.38
	9-17-48									
	9-27-48	c	10	F	Methionine (50 mg)	6.1 ± 0.41	17.6 ± 2.2	140 (117-158)	—10.9 ± 2.2	4.5 ± 0.21
	to	d	10	F	Eth. est. (30 γ) + Methionine (50 mg)	5.5 ± 0.22	8.9 ± 1.6	140 (122-160)	—13.5 ± 1.5	4.4 ± 0.18
	1-17-49									

In experiments 24 and 25, the rats were pair fed.

the lipotropic effect of ethinyl estradiol *plus* methionine appeared to be more pronounced in Fischer rats than seen before[1,2] with rats of genetically different and less homogeneous strains. For instance, in one experiment (Table I, Exp. 24), the average fat content of the liver in the control animals was found to be 33.1%, in the group treated with methionine, 18.7%, whereas in rats treated with methionine *plus* ethinyl estradiol, the perfectly normal figure of 5.7% was reached. It should be kept in mind that in these experiments the animals were pair-fed.

In histological examination§ the testes of rats receiving estrogen showed signs of degeneration with consecutive atrophy. In the control groups the testes exhibited essentially normal histological findings. In female rats receiving estrogens constant estrus was observed in contrast to the control rats and to the animals treated with methionine only, which showed essentially normal estrous cycles.

The exceptionally marked response of rats of the Fischer strain to an alipotropic diet is in good accord with the recently published behavior of the same strain with regard to methionine uptake by liver slices.[5] Compared with an inbred Wistar strain, liver slices obtained from Fischer rats showed a very low uptake of methionine. These observations together with the results here presented should reemphasize the importance of genetic variations in stock laboratory animals when used in this type of experiment.

Conclusions. 1. The fat infiltration of the liver in response to an alipotropic diet is more uniform and more intensive in rats of the inbred Fischer strain than that seen in the past in genetically less homogeneous strains. 2. Estrogen, in particular ethinyl estradiol, when given in combination with methionine, exerts a very marked lipotropic effect with a corresponding reduction of the liver fat to normal values.

§ We are indebted to Dr. Harry Goldblatt (Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.) for the histological examination of the testes.

[5] Rutman, R., Dempster, E., and Tarver, H., *J. Biol. Chem.*, 1949, **177**, 491.

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17254. Effects of Pituitary Adrenocorticotropic Hormone (ACTH) in Children with Non-Addisonian Hypoglycemia.

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Severe chronic hypoglycemia of the non-Addisonian type presents a difficult etiologic and therapeutic problem, unless by good fortune it is found on direct examination of the pancreas to be due to a removable adenoma of the islet cells in that organ, a comparatively rare pathological condition. Dietary control of the blood sugar level, which may be quite successful in some cases of mild "functional" or "recurrent" hypoglycemia, is ineffectual in the severe persistent type. Surgical removal of as much as 80 to 85% of the histologically normal pancreas, a therapeutic procedure of last resort, is likely to give temporary relief only, as in two members of the series of patients included in the present investigation. Cautious use of alloxan in one severe case of idiopathic hypoglycemia appeared to be successful.[1] Despite its hepatotoxic action, this agent deserves further trial but only under the strictest supervision.

Repeated attempts on our part to force the gluconeogenetic action of cortin to a degree sufficient to control non-Addisonion hypoglycemia, while midly encouraging in a few instances, have been unsuccessful in the main. However, the isolation of pituitary adreno-

[1] Talbot, N. B., Crawford, J. D., and Bailey, C. C., *Pediatrics*, 1948, **1**, 337.