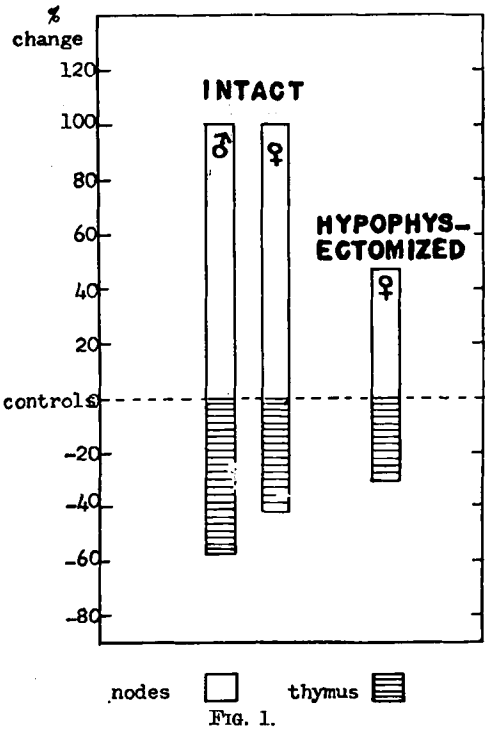

FIG. 1.

Per cent change over control values of thymus and lymph nodes weights expressed as mg per 100 g of body weight. The columns on the left refer to the intact animals; the one on the right refers to the hypophysectomized mice.

possibility of the hyperplasia being due to infection, regional lymphadenopathy or metastases of the tumor has been excluded in an earlier series by histological studies and a true lymphatic hyperplasia established by the clear cut increase in nitrogen content of these nodes.[20] There was thus, under the conditions of the experiment, a distinct dissociation of the response of the thymus and of the lymph nodes to the presence of growing Sarcoma 180.

In view of the fact that the growth of Sarcoma 180 in the CFW strain of mice is accompanied by adrenal changes indicative of an increased stimulation of the latter organ by the anterior pituitary[21] the possibility of a relationship between the endocrine system and the lymphatic and thymic changes was considered. The results of the second experiment shown in Table II indicate however that in the hypophysectomized animal, the growing tumor evoked a thymic atrophy and lymphatic hyperplasia, though to a lesser degree than in the intact animal; the adrenal hypertrophy and lowered ascorbic acid levels induced by the tumor in the intact animal[21] were, on the other hand, not observed in the hypophysectomized mice.[23] It is thus implied that the thymic and lymphatic changes are not mediated through the pituitary-adrenal system.

Summary. Transplanted Sarcoma 180 after 12 days of growth evokes thymic atrophy and lymphatic hyperplasia in mice of the CFW strain. These changes are not prevented by hypophysectomy, thereby excluding pituitary-adrenal mediation.

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Response of Guinea Pigs to Diets Deficient in Choline.

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The hepatic choline concentration of the choline-deficient rat, when calculated on a fat-free basis, is not lower than that of normal rats.[1,2] This fact has been correlated with the observation that the choline oxidase activity of the fatty livers of choline deficient rats is markedly suppressed[3] and the suggestion advanced that it is the diminished choline

1 Jacobi, H. P., and Baumann, C. A., *J. Biol. Chem.*, 1942, **142**, 65.

2 Handler, P., and Dann, W. J., *J. Biol. Chem.*, 1942, **146**, 357.

3 Handler, P., and Bernheim, F., *J. Biol. Chem.*, 1942, **144**, 401.

TABLE I.
Composition of Diets.

	1	2	3	4	5	6	7
Casein	12	12	12	12	10	6	6
Cellulose	20	20	10	20	20	15	10
Salts[18]	4	4	4	5	5	5	5
Liver "L"	3	3	3	5	5	5	4
Lard	10	10	15		20	15	25
Glucose	48	48	52	55			
Sucrose					23	21	15
Cod liver oil	3	3	3	1			
Cystine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cholesterol	0.3	0.3	0.3	0.3	0.5	0.5	0.5
Inositol	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glycocyamine	0.1	0.3	0.3	0.3	0.5	0.5	0.3
Arginine				1.0	1.0	1.0	0.5
Glycine				0.3	0.3	0.3	0.3
Peanut Meal						30	30
Cerophyl							3

oxidase activity which permits the existence of a normal hepatic choline concentration (as phospholipid) despite the dietary choline deficiency.[1,3] Choline deficiency and its manifestations have been most frequently studied in the rat[4] but have also been observed in the dog,[5] mouse,[6] and chick[7] as well as implicated in the etiology of hepatic cirrhosis in man. The livers of these species have all been shown to possess marked choline oxidase activity.[8] Although fatty livers have frequently been observed in the guinea pig, under various conditions there has been, as yet, no demonstration that these have been, in any case, the result of dietary choline deficiency. Since the liver of this species is unique among common laboratory animals in that it possesses little or no choline oxidase activity,[8] it was of considerable interest to determine the susceptibility of this species to dietary choline deficiency. The present paper describes a number of attempts to elicit some manifestation of choline deficiency in young guinea pigs under conditions which were quite effective for the rat.

Experimental. In preliminary studies various procedures for the feeding of synthetic rations to young guinea pigs were investigated. In some instances the newborn pigs were offered the experimental diets while still suckling and were continued on them after separation from the sow. A few attempts were made to offer such rations to one-week-old pigs weaned at that time. However, the data to be presented were all obtained in the following manner. Weanling guinea pigs were immediately offered a diet consisting of equal parts of the synthetic ration and minced carrots. After 4 days the amount of carrots was reduced by half and 4 days later the carrots were removed entirely. A group of rats, as controls, was also offered each of the experimental rations in similar fashion. All animals were housed in individual cages with food and water available *ad libitum.* Three weeks after deletion of the carrots from the diet the animals were sacrificed by decapitation and their livers taken for lipid analysis.[9]

Using this feeding technic relatively few young pigs refused the experimental rations. All animals which failed to eat adequately or which lost weight unduly and died before termination of the feeding trial were discounted in evaluating the results of these experiments. This was considered valid since approximately equal numbers of pigs died on both choline-deficient and choline-supplemented rations. It is of interest that, in many instances, on each of the experimental rations employed, pigs died suddenly after a

4 Best, C. H., and Lucas, C. C., *Vitamins and Hormones,* 1943, **1**, 1.

5 Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.,* 1933, **79**, 94.

6 Handler, P., unpublished data.

7 Jukes, T. H., *J. Nutrition,* 1940, **20**, 445.

8 Bernheim, F., and Bernheim, M. L. C., *Am. J. Physiol.,* 1938, **121**, 55.

9 Handler, P., *J. Biol. Chem.,* 1948, **173**, 295.

TABLE II.
Liver Lipids of Guinea Pigs and Rats on Choline-Deficient Diets.

Group	Species	No. animals	Diet	Initial wt, g	Wt change, g	Food intake, g/day	Liver lipids, % wet wt
1	GP	6	1	188	— 7	9.3	4.8
2	"	5	1 + choline	181	— 6	10.0	4.6
101	Rat	6	1	82	29	8.3	11.6
102	"	6	1 + "	76	31	8.1	5.3
3	GP	5	2	189	2	9.2	4.4
4	"	6	2 + "	196	— 3	9.6	4.1
103	Rat	5	2	68	26	7.7	13.8
104	"	5	2 + "	73	23	7.9	6.1
5	GP	7	3	161	—11	9.1	4.3
6	"	6	3 + "	173	— 6	9.9	4.4
105	Rat	6	3	84	16	7.2	15.6
106	"	6	3 + "	76	24	7.9	5.9
7	GP	5	4	143	9	8.7	4.9
8	"	8	4 + "	148	4	8.4	5.2
107	Rat	6	4	61	29	8.0	10.8
108	"	6	4 + "	60	27	8.0	4.7
9	GP	6	5	148	—13	6.9	5.6
10	"	6	5 + "	156	—17	7.1	5.3
109	Rat	5	5	69	17	6.3	21.7
110	"	5	5 + "	64	23	6.1	6.3
11	GP	7	6	173	41	12.7	7.4
12	"	6	6 + "	184	37	11.9	7.7
111	Rat	6	6	67	16	6.7	24.2
112	"	6	6 + "	72	23	6.6	5.8
13	GP	6	7	177	47	11.8	8.3
14	"	6	7 + "	189	41	11.4	6.9
15	"	5	7 + methionine	180	40	11.5	8.2
113	Rat	5	7	74	14	7.0	26.7
114	"	5	7 + choline	61	19	6.9	6.8

variable period of reasonable weight gain or maintenance and apparently adequate food consumption. Similar deaths have not been observed in animals living on the stock diets commonly employed for this species.

The various diets employed are summarized in Table I. Each diet was offered to a group of control rats as well as to guinea pigs. To each kilo of each diet was added thiamine 10 mg, riboflavin 12 mg, pyridoxine 10 mg, *p*-aminobenzoic acid 25 mg, calcium pantothenate 50 mg, niacin 50 mg, ascorbic acid 200 mg, α-tocopherol 100 mg, and 2-methyl-1, 4-naphthoquinone 10 mg. All animals received 50 γ biotin and 50 γ folic acid 3 times weekly by pipette. All animals not receiving cod liver oil in the diet were given 2 drops of percomorph oil 3 times weekly. All animals on diets 3 - 7 were given 1 drop of cream 3 times weekly by pipette. Not indicated in the tables is the fact that each of these diets was also fed without inositol. However, in no case did the absence of this substance appear to affect the results. Choline, when present was incorporated as 0.6% of the diet as was DL-methionine. The results are summarized in Table II.

The inclusion of liver fraction "L" in all diets was based on the findings of Woolley and Sprince[10] and has been found to contain but 0.87 mg of choline per gram. Arginine and glycine were added to diets 4 - 7 as these have been found to improve the growth of guinea pigs on low protein rations.[11] Glycocyamine was added to diets 2 - 7 since, like nicotinamide, it forces a demand for methyl groups

[10] Woolley, D. W., and Sprince, H., *J. Biol. Chem.*, 1944, **153**, 687.

[11] Woolley, D. W. personal communication.

and increases the choline and/or methionine requirement of the rat. [12] "Cerophyl" was included in diet 7 as a source of "grass juice factor". The rations containing alcohol-extracted peanut meal were patterned after those used by Engel and Salmon[13] and proved to be the most successful diets in this study. It will be seen that most of the guinea pigs on diets 1 - 5 grew very poorly or not at all and a considerable number actually lost weight during the experimental period. It has been noted that under such conditions little or no fat accumulates in rat livers despite choline deficiency[2,14,15] and it is now well recognized that the optimal conditions for eliciting the hepatic and renal manifestations of choline deficiency are those which permit the maximum growth rate compatible with the relatively low protein diets necessary in such studies.[16] The peanut meal rations 6 and 7 much more satisfactorily met these requirements as seen in Table II.

It is apparent that while diets 1 - 5 were sufficiently lacking in choline and methionine to produce fatty livers in rats, they did not result in the accumulation of fat in guinea pig livers. Diets 6 and 7, which were the most effective of this series in producing fatty livers in rats, also resulted in a slight elevation of the fat content of the guinea pig livers. However, this was not diminished by the inclusion of choline or methionine in these diets and, consequently, this effect cannot be ascribed to choline deficiency.

The data presented herein should not be interpreted as indicating that the metabolism of phospholipids in the guinea pig is radically different from events in the rat. The possibility exists that the conditions of these experiments permitted sufficient intra-intestinal synthesis of labile methyl compounds to meet the animals' requirements. However, the failure of glycocyamine, like that of nicotinamide,[17] to increase the demand for a supply of methyl groups sufficiently to result in fatty liver formation is surprising. The present findings are compatible with the lack of hepatic choline oxidase activity in this species and suggest that the lack of this enzyme results in a choline turnover so slow as to permit a nutritionally adequate supply of choline from such choline and methionine as was available in the diets of this study. While it may yet be found that the position of choline in the nutrition of the guinea pig is much like that in the rat, the daily requirement of the guinea pig for choline (or its equivalent in methyl groups) is certainly of a lower order of magnitude than that of the rat.

Summary. Seven different choline deficient diets were fed to young rats and guinea pigs. While each of these diets resulted in fatty liver formation in the rats, in no instance was there observed an appreciable accumulation of fat in the guinea pig livers. This fact has been correlated with the lack of hepatic choline oxidase activity in the guinea pig as compared to all species which have, to date, been found susceptible to dietary choline deficiency.

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[12] Stetten, DeW., and Grail, G. F., *J. Biol. Chem.*, 1942, **144**, 175.

[13] Engel, R. W., and Salmon, W. D., *J. Nutrition*, 1941, **22**, 109.

[14] Handler, P., *J. Biol. Chem.*, 1943, **149**, 291.

[15] Handler, P., *J. Biol. Chem.*, 1946, **162**, 77.

[16] Griffith, W. H., *Biol. Symposia*, 1941, **5**, 193.

[17] Handler, P., *J. Biol. Chem.*, 1944, **154**, 503.