known positive washings. While technically more difficult, the inoculation of penicillin and throat washing into the amniotic sac proved to be a highly satisfactory method of detecting influenza A virus and was the most sensitive of present-day methods in this experiment.

14880

Influence of Protein and Thiamine Intake on Pathologic and Weight Changes Produced by Atabrine.*

HENRY SIEGEL, CHARLES W. MUSHETT, AND GLADYS A. EMERSON.
From the Merck Institute for Therapeutic Research, Rahway, N.J.

The oral administration of atabrine produces necrosis and replacement fibrosis of the liver and myocardium in the rat.[1-3] A recent report[4] indicates that the toxic effects of atabrine upon the liver, as measured by an increase in plasma fibrinogen, can be largely prevented by a high protein-low fat diet. The present investigation was undertaken to determine the effect of the protein and thiamine content of the diet upon the hepatic and myocardial lesions in the rat which result from atabrine administration. The concomitant effect upon growth was also studied.

Experimental. Effect of Protein Intake. Young male rats of the Carworth strain were segregated into 8 groups of like average weight (123 g) and were maintained on high or low protein diets. In addition several of the groups received atabrine as indicated in Fig. 1. Four rats were employed in each of the control groups and 20 rats in each of the groups receiving atabrine.

The two experimental diets used had the following composition:

Low Protein—vitamin free casein, 6; dextrose, 78; hydrogenated vegetable fat, 8; cod liver oil, 2; mazola, 1; dried beef liver, 1; salt mixture USP XI No. 1, 4; supplemented with 0.8 mg each of thiamine, riboflavin, and pyridoxine; 8.0 mg each of nicotinamide and calcium pantothenate and 100 mg of choline chloride per 100 g of diet.

High Protein—This diet was identical with the low protein diet except that 24% of the dextrose was replaced by 24% casein, thus raising the casein content to 30% and lowering that of the dextrose to 54%.

The rats were allowed to equilibrate on these diets for a period of one week prior to dosing with atabrine. All animals receiving the drug were dosed daily by stomach tube with 45 mg per kg (5% of the L.D. 50). The experiment was terminated after 48 days (41 days of atabrine administration), at which time the rats on the low protein diet were in a debilitated condition.

At autopsy the degree of hepatic damage was estimated grossly by the amount of the entire liver which was necrotic. The heart, after fixation in 4% formaldehyde solution, was cut in half by frontal section. Histologic sections were made parallel to the cut surfaces of both halves and stained with hematoxylin-eosin. The degree of myocardial damage was graded as slight or moderate. Rats with "slight" involvement had only an occasional focus of necrosis; in those with "moderate" involvement several areas of the myocardium were affected.

Atabrine administration had a marked depressing effect upon weight as evidenced by the difference in the growth of rats in Groups

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Merck Institute for Therapeutic Research.

1 Wright, C. I., and Lillie, R. D., *Public Health Rep.*, 1943, **58**, 1242.

2 Fitzhugh, O. G., and Nelson, A. A., *Fed. Proc.*, 1944, **3**, 72.

3 Siegel, H., and Mushett, C. W., *Arch. Path.*, 1944, **38**, 63.

4 Scudi, J. V., and Hamlin, M. T., *J. Pharm. and Exp. Therap.*, 1944, **80**, 150.

TABLE I.
Degree and Extent of Liver and Myocardial Damage of Atabrine Dosed (45 mg per kg) Male Rats on High and Low Protein Diets.*

Group	No. of rats	Diet	Liver damage		Myocardial damage	
			% with necrosis	Avg degree of involvement	% with necrosis and fibrosis	Avg degree of involvement
2	21	Low protein *ad lib.*	95	severe	48	slight
5	18	High protein *ad lib.*	44	slight	83	slight to moderate
8	19	High protein restricted to group 2	84	slight to moderate	79	moderate

* The livers and hearts were normal in all *ad libitum* as well as restricted diet controls.

1 and 2 and in Groups 4 and 5 (Fig. 1). Furthermore, the animals in each of the atabrine-dosed groups weighed less than did their isocaloric controls (Groups 2 and 3; 5 and 6). Rats on the high protein diet *ad libitum* which were dosed with atabrine showed a greater depression in weight relative to their undosed isocaloric controls (43 g) than did the corresponding rats on the low protein diet (23 g).

The incidence and degree of myocardial necrosis and replacement fibrosis after atabrine administration were greater in the animals fed the high protein diet than in those fed the low protein diet (Table I). On the other hand, the highest incidence and degree of liver necrosis were observed in the animals receiving the low protein diet (Group 2, Table I.). Although a high percentage of the rats on the high protein diet, restricted in caloric intake to that of the low protein animals (Group 8), showed liver necrosis, the degree of involvement was less than that in the latter group. The rats receiving the high protein diet *ad libitum* (Group 5) showed the lowest incidence and degree of hepatic damage. The livers and hearts of all controls (not receiving atabrine) were normal.

Effect of Thiamine Intake. In another experiment the effects of a thiamine-low diet upon the toxicity of atabrine were studied. Adult rats maintained on 2 to 4 micrograms thiamine daily, were as resistant as were their controls to the pathological changes induced by atabrine administration (45 mg per kg).

Discussion. It has been shown by Goldschmidt, Vars and Ravdin[5] that a low protein diet will render the liver more susceptible to hepatoxic substances. More recently, Scudi and Hamlin[4] have demonstrated that a low protein diet aggravates the degree of atabrine toxicity in rats and dogs as measured by an augmentation in plasma fibrinogen, the values of which parallel closely the degree of

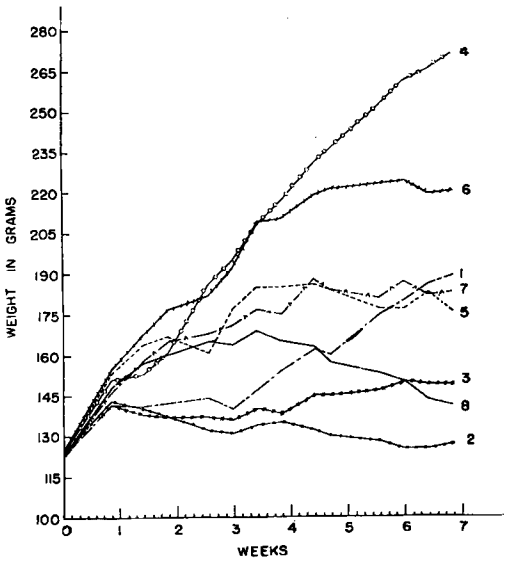

FIG. 1
Weight curves of groups of rats on high and low protein diets with and without atabrine.
Group 1—Low protein diet ad lib.
Group 2—Low protein diet ad lib. plus atabrine (45 mg/kg daily)
Group 3—Low protein diet; caloric intake restricted as in Group 2
Group 4—High protein diet.
Group 5—High protein diet ad lib. plus atabrine (45 mg/kg daily)
Group 6—High protein diet; caloric intake restricted as in Group 5
Group 7—High protein diet; caloric intake restricted as in Group 2
Group 8—High protein diet; caloric intake restricted as in Group 2 plus atabrine (45 mg/kg daily)

5 Goldschmidt, S., Vars, H. M., and Ravdin, I. S., *J. Clin. Invest.*, 1939, **18**, 277.

hepatic damage in the rat.[6] In the studies herein reported a high level of dietary protein appeared to have a protective effect against the hepatic necrosis produced by atabrine (45 mg per kg). Hegsted, McKibbin and Stare[7] failed to observe hepatic necrosis or myocardial damage in rats receiving 40 mg % atabrine in the diet for a period of 6 months. The intake of the drug, however, was probably less than 40 mg per kg body weight due to the depressing effect of the compound upon appetite.

A low protein diet failed to aggravate the toxic effect of atabrine upon the muscle of the heart. On the contrary, the incidence and degree of myocardial damage were higher in the animals receiving a high protein diet, a finding in agreement with that of Nelson and Fitzhugh.[8]

The pathological changes produced by atabrine in the heart and in the liver were the same in rats maintained on a low thiamine diet as in those on a natural food ration. In this connection it is of interest to note that Follis[9] found that a thiamine deficiency was protective against the myocardial lesions produced by a deficiency in potassium. Hegsted *et al.*[10] reported that atabrine has a "thiamine sparing" action when fed to rats on a thiamine deficient ration.

The influence of other dietary constituents upon atabrine toxicity has recently been reported. Wright and Lillie[1] found that the tolerance to atabrine was not influenced by the addition of riboflavin to a stock ration. Hegsted, *et al.*[7] reported that the slow growth obtained on sub-optimal levels of riboflavin was further decreased by the addition of 40 mg % atabrine to the diet. On the other hand, these authors found that with diets sub-optimal in vitamin A, the feeding of atabrine did not cause a further reduction in growth rate. Hegsted *et al.*[11] have also reported that the inclusion of atabrine in low choline diets at a level of 65 mg per 100 g of ration almost completely prevents hemorrhagic kidneys in the rat.

Summary. The hepatic damage resulting from continued atabrine administration was more severe in rats maintained on a low protein diet than in those on a high protein diet. On the other hand, the incidence and degree of myocardial damage were slightly greater in the rats fed the high protein diet. Thiamine deficiency had no effect upon the hepatic and myocardial damage produced by atabrine. The daily administration of atabrine to rats maintained on high and low protein rations resulted in a retardation of growth beyond that observed in their isocaloric controls which did not receive atabrine.

6 Silber, R. H., Clark, I., and Siegel, H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 115.

7 Hegsted, D. M., McKibbin, J. M., and Stare, F. J., *J. Nutrition*, 1944, **27**, 141.

8 Nelson, A. A., and Fitzhugh, O. G., *Fed. Proc.*, 1944, **3**, 91.

9 Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1942, **71**, 235.

10 Hegsted, D. M., McKibbin, J. M., and Stare, F. J., *Fed. Proc.*, 1944, **3**, 94.

11 Hegsted, D. M., McKibbin, J. M., and Stare, F. J., *J. Nutrition*, 1944, **27**, 149.