of vitamin B_6—pyridoxal, pyridoxamine, pyridoxine and pyridoxic acid—were measured in normal human urine and in the urine of human subjects each fed one of the 3 forms of the vitamin.

The chief product found, regardless of the form fed, was pyridoxic acid. Pyridoxal gave rise to significantly higher amounts of this product than did pyridoxine or pyridoxamine. No evidence could be obtained showing the conversion of pyridoxal or pyridoxamine to pyridoxine. When pyridoxal or pyridoxine was fed, the chief form in which the vitamin occurred in the urine was the form fed. However, when pyridoxamine was fed both pyridoxal and pyridoxamine were excreted in approximately equal amounts. Ingestion of pyridoxine also greatly increased the amount of pyridoxal and pyridoxamine excreted.

The excretion of all products was very rapid. The largest amounts of each of the compounds were found in samples collected 2 and 5 hours after ingestion of the dose. The levels of pyridoxic acid returned to normal values after 12 hours, while the vitamin levels had returned to normal within 8 hours. The amount of the dose recovered varied with the form fed. The highest recovery, 70%, was obtained when pyridoxal was fed; 45% of the pyridoxine was recovered, while only 31% of the pyridoxamine could be recovered. Together with published data which indicate that complete absorption of large doses of vitamin B_6 occurs, these findings suggest that a large proportion of the vitamin B_6 was converted to products still unknown.

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Loss of Body Protein and Antibody Production by Rats on Low Protein Diets.*

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(Introduced by Paul R. Cannon.)
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Previous studies in this laboratory have demonstrated that prolonged severe deficiency of protein without other dietary restriction results in a reduced capacity of the rat to produce antibodies to sheep erythrocytes,[1] Friedlander's bacillus,[2] pneumococci,[3] and most recently a parasitic nematode.[4] Concomitant with the reduction of antibody production there was shown to be a reduction in the capacity to form leukocytes of both the granulocytic and lymphocytic series.[5] Associated with these phenomena there is a reduction in the resistance of animals to infection with virulent organisms.[3] Furthermore this reduction in resistance was shown to be due largely to the inability of the animals to fabricate antibodies since the depleted animals, when passively immunized, survive the infection as well as the normally nourished controls. Having established the fact that protein depletion of long duration reduces the rate of antibody formation and the resistance to infection it then becomes of interest to investigate the rate of decay of the antibody forming capacity with time under conditions

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1 Cannon, P. R., Wissler, R. W., Woolridge, R. L., and Benditt, E. P., *Ann. Surg.*, 1944, **120**, 514.

2 Woolridge, R. L., unpublished observations.

3 Wissler, R. W., *J. Infect. Dis.*, 1947, **80**, 264.

4 Woolridge, R. L., unpublished observations.

5 Asirvadham, M., *J. Infect. Dis.*, 1948, **83**, 87.

of dietary protein deficiency and to attempt to relate this process to the loss of protein from various body compartments.

Materials and methods. The subjects of the experiments were young adult male albino rats (Sprague-Dawley). On arrival at the laboratory the animals were housed 7 to a cage and fed Wayne Dog Chow until they had reached a weight between 200 and 220 g. They were then started on the low protein regime. The detailed composition of the low protein diet (3E) has been described elsewhere.[6] The ration used in the present experiment contained corn oil as the fat. The content of all dietary essentials in this diet is sufficient to support normal growth when an adequate protein supplement is added. For the first 30 days of the depletion period the animals were offered 15 g of the ration each day. After 30 days the quantity was increased to 20 g per animal per day. Diet consumption is practically complete with this diet until more than 25% of the body weight has been lost after which diet intake slowly falls. The most depleted animals in this experiment never ate less than 72% (14.4 g) of their diet on the average.

In this study groups of 5 rats having comparable weights prior to depletion were selected from larger lots which had been on the depletion regime for 0, 17, 32, 43, 56, and 100 days. At the start of the final period of the experiment the animals were placed in individual cages and fed the same low protein diet for the last 11 days. On the day they were placed in the individual cages each animal was given by tail vein an injection of one ml of a 0.25% suspension of 5 times washed sheep's erythrocytes, and one ml of a heat killed vaccine of Friedlander's bacillus containing 1.6 billion organisms per milliliter. Six days after the injection of antigen, blood volumes, hematocrits, hemoglobins, serum protein concentrations and agglutinin and hemolysin titers were determined.[7] Five days following this the animals were sacrificed. The

TABLE I.* Protein Content Several Body Compartments and Rate of Antibody Formation in Rats on Low Protein Diet for Various Periods of Time.

Depletion to sacrifice, days	No. of animals	Live wt at sacrifice, g	Carcass protein, g	Total circulating serum protein, mg	Liver protein, g	Total circulating hemoglobin, mg	Depletion at time of inj., days	Agglutinin titers	Hemolysis titer
0	5	206 ± 1.6	32.1 ± .58		1.34 ± .063				
11	5	177 ± 2.1	30.8 ± .33	514 ± 19	1.07 ± .038	2370 ± 36	0	3840 ± 0	7680 ± 0
28	5	171 ± 2.2	31.0 ± 0.22	410 ± 7	1.25 ± .067	1900 ± 94	17	2200 ± 300	6700 ± 940
43	5	169 ± 3.7	30.3 ± 0.60	390 ± 10	0.91 ± .047	1410 ± 79	32	1280 ± 220	1920 ± 0
54	5	161 ± 1.9	30.7 ± 0.75	380 ± 6	0.84 ± .042	1490 ± 61	43	960 ± 0	1920 ± 390
67	5	147 ± 1.4	29.1 ± 0.60	341 ± 12	0.73 ± .021	1290 ± 61	56	320 ± 90	1280 ± 370
111	4	129 ± 4.6	23.2 ± 1.00	266 ± 15	0.70 ± .041	1150 ± 69	100	18 ± 3	340 ± 70

* Values are given as means and standard errors for groups of animals.

[6] Wissler, R. W., Woolridge, R. L., Steffee, C. H., and Cannon, P. R., *J. Immunol.*, 1946, **52**, 267.

[7] Benditt, E. P., Straube, R. L., and Humphreys, E. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 189.

carcasses and livers were analyzed for fat, water and protein by methods which have been described.[8] Sections were made of the livers and these in conjunction with the detailed liver analyses are the subject of another report.[9]

Observations. The observations are summarized in Table I. In Fig. 1 are plotted the

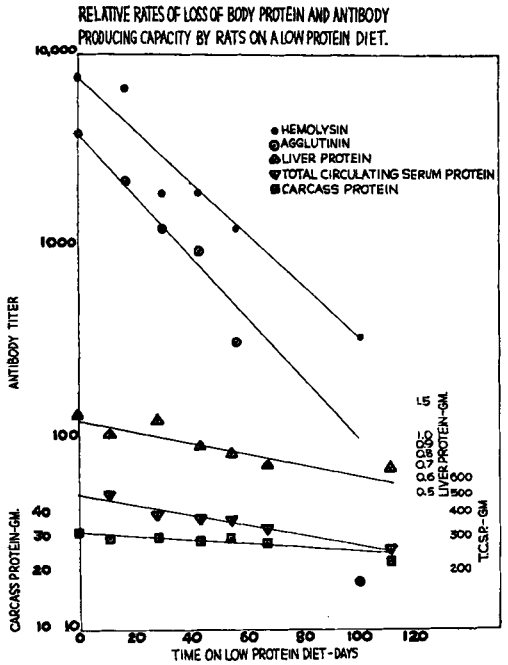

FIG. 1.

data for carcass protein, liver protein, total circulating serum protein, and the antibody titers (agglutinins and hemolysins). They are plotted on a semilogarithmic scale with time on the linear axis. Such a chart allows one to compare the relative rates of decline of these various factors by comparing the slopes of the curves.

To a reasonable first approximation the loss of protein from the carcass, liver, serum and erythrocytes occurs at a constant percentage rate. The same is true for the loss of the capacity to form antibody. The daily percentage rates of decline as estimated from these curves are: Carcass, 0.3; total circulating serum protein 0.6; liver protein, 0.7; rates of antibody production, antibacterial agglutinins), 3.7; and (anti-sheep erythrocyte hemolysin), 3.0. The rate of loss of hemoglobin is approximately the same as for serum protein. Liver and plasma proteins decline at approximately equal rates, and approximately twice as fast as the carcass protein. The antibody forming capacity declines 10-13 times as fast as the carcass protein content.

Statistical analysis shows that the decrease in hemolysin titer is not significant until after 30 days following initiation of the low protein diet. The agglutinin titers appear to be just significantly diminished ($p < .05$) by the 17th day of the depletion regime.

Discussion. Recently Metcoff and co-workers[10] have questioned the role of dietary protein deficiency in impairment of antibody formation. These investigators injected young rats with *S. typhimurium* 6 days after initiation of the low protein diet. As might be expected, they could find no difference in antibody titers up to 28 days from the start of the protein deficiency. On the 28th day they injected typhoid "O" antigen and 14 days later removed blood for titers in an attempt to demonstrate an "anamnestic" response. From their experience these authors conclude "that dietary protein deficiency does not seriously impair the mechanism for protective antibody formation in the rat."

The present observations demonstrate clearly that severe restriction of the dietary protein intake, despite the presence of an adequate supply of other essential nutrients causes marked impairment of antibody production. Further they demonstrate that the capacity to form antibody decreases with time on the low protein diet but the decrease does not become appreciable with some antigens until several weeks or more have elapsed. Whipple and his co-workers[11] early recognized that many weeks of protein depletion were

8 Benditt, E. P., Humphreys, E. M., Wissler, R. W., Steffee, C. H., Frazier, L. E., and Cannon, P. R., *J. Lab. and Clin. Med.*, 1948, **33**, 257.

9 Jaffe, E. R., Humphreys, E. M., Benditt, E. P., and Wissler, R. W., *Arch. Path.*, in press.

10 Metcoff, J., Darling, D. B., Scanlon, M. H., and Stare, F. J., *J. Lab. and Clin. Med.*, 1948, **33**, 47.

11 Madden, S. C., and Whipple, G. H., *Physiol. Rev.*, 1940, **20**, 194.

necessary to exhaust the "protein reserves" of their animals and reduce the rate of plasma protein fabrication significantly.

Since antibody formation appears to be influenced by dietary protein intake it seems likely in general that anything which interferes substantially with protein metabolism such as caloric inadequacy[8] for a sufficient length of time will also impair antibody formation.

One further point is apparent in the data of this experiment. The protein *content* of any body compartment is an insensitive indicator of the rate of turnover or fabrication of protein in the compartment. In Fig. 1 it can be seen that when the total circulating serum protein fell to about 50% of its initial value the rate of antibody production has fallen to 1% or less of its initial value. From previous experiments[12] we know that the circulating quantity of that portion of the globulin containing the antibody is reduced only to an equal or lesser extent than the total protein, *i.e.* 50% or less. Practically this means that the measurement of such quantities as serum protein concentration, total circulating serum protein or any one of its components may give little if any insight into the *rate* of production or turnover of the protein.

Summary. The present experiment investigated the rate of loss of the capacity to produce antibody in comparison with the rate of loss of protein from body compartments including carcass, liver and plasma. Young adult male albino rats were tested at intervals from 0 to 100 days after initiation of the low protein diet for agglutinin and hemolysin production. Blood volumes, hemoglobin, serum protein, liver and carcass protein were determined. It was found that all protein compartments and the rate of antibody production fell at approximately linear percentage rates per day, the antibody production falling much faster than the rest. Statistically significant depression of the antibody titers was not reached till the 30th day with hemolysins and the 17th day with the agglutinins.

[12] Benditt, E. P., unpublished observations.

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Chemotherapy of *Trichomonas foetus* Infections in Rabbits.*

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No satisfactory therapeutic agent has been established for the successful treatment of bovine trichomoniasis. *In vivo* studies in laboratory animals of the trichomonacidal properties of various chemicals may offer useful information for future treatment trials in cattle. The purpose of this paper is to present the results of a study on the effect of 58 compounds on *Trichomonas foetus* in the vagina of rabbits. The majority of the compounds were selected from 350 chemicals previously tested *in vitro* by Morgan and Campbell.[1]

Witte[2] apparently was the first to successfully infect rabbits with *T. foetus* by vaginal

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† Wellcome Research Fellow of the Animal Health Trust (from the Ministry of Agriculture and Fisheries Laboratories, Weybridge, England).

[1] Morgan, B. B., and Campbell, H. M., *Am. J. Vet. Res.*, 1946, **7**, 45.

[2] Witte, J., *Arch. wiss. prakt. Tierheilk.*, 1933, **66**, 333.