6795

Nitrogen Metabolism in White Mice in the Course of Tumor Development.

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Data were presented concerning the composition of the urine of normal white mice.[1, 2] Attention was drawn[2] to the fact that the urine of white mice is remarkably concentrated, average specific gravity 1.068, and to the uniformly high content of a protein of a chondromucoid type.

In the course of a biochemical study of the development of transplanted tumor in white mice studies were made of the composition of the urine and the data compared with that of urine from normal mice. The results obtained are described below.

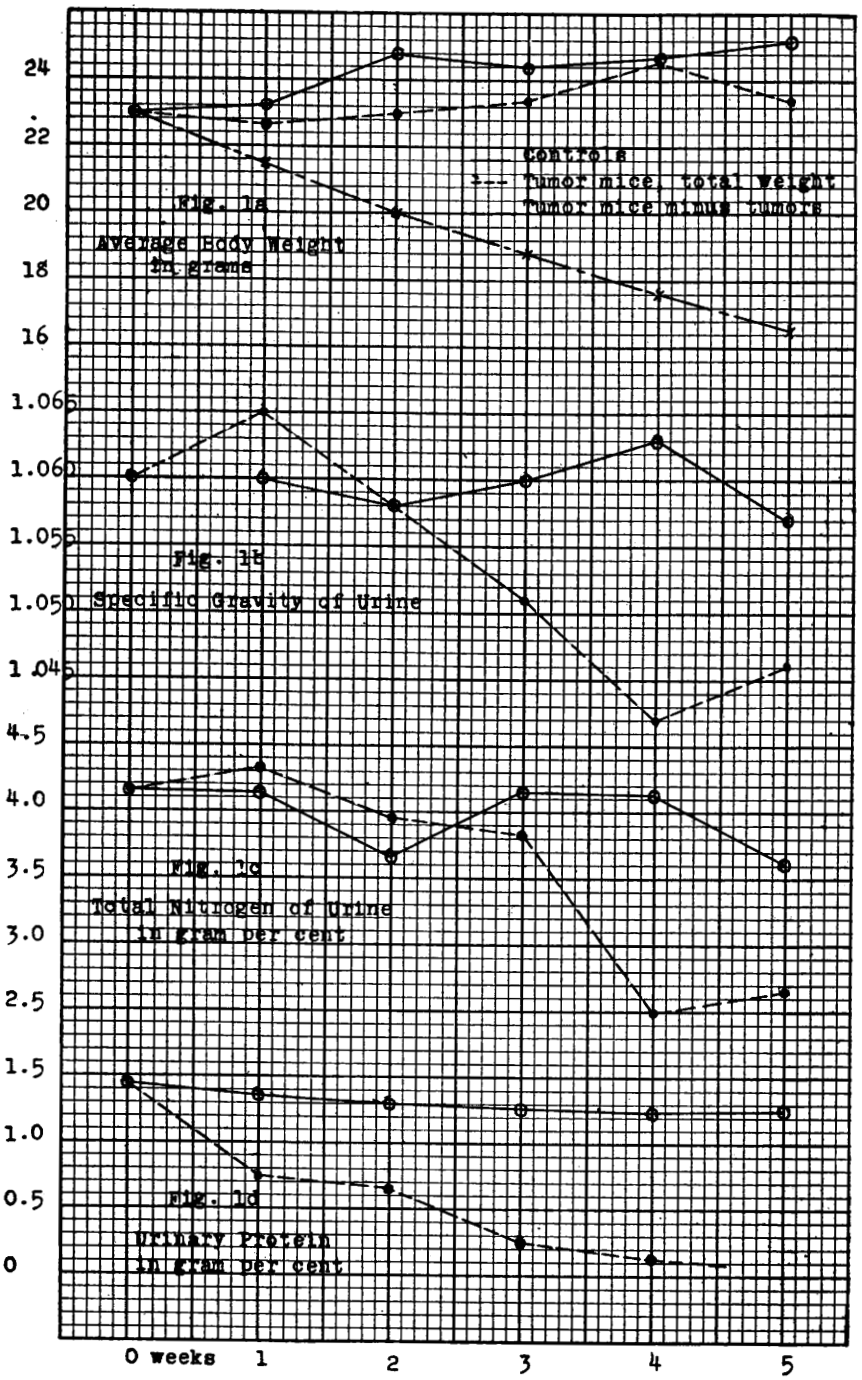
Three hundred tumor mice and 400 control animals were used. The animals were approximately of the same age and source in both groups, were kept under the same conditions and given the same diet of oats, bread and water.

The tumor material used was Sarcoma 180 of the Crocker Institute. It was transplanted subcutaneously in the usual manner into young adult mice. The virulence of this tumor is uniformly great, producing 95 to 100% takes, and most of the mice die within 6 weeks after the implantation. In most instances by the end of the process the tumors reached relatively enormous proportions with an area of 12 sq. cm., and weighing about one-third of the total body weight. Quite frequently, however, the mice died with much smaller tumors. The total body weight of the mice, including the tumors, remained quite stationary and equal to that of the controls, as shown in Fig. 1a. But the true body weight obtained after excising the tumor declined steadily from the average value of 23 gm. to 16 gm., amounting to a loss of about one-third of tissue substance, which was evidently used for the growth of the tumor.

The urine was obtained directly from the bladder by gentle massage and pooled samples from about 50 animals at a time were analyzed by the methods stated in the previous paper.[2] The tumor mice yielded uniformly less urine than the normal animals. The

[1] Parfentjev, I. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1285.

[2] Parfentjev, I. A., and Perlzweig, W. A., *J. Biol. Chem.*, 1933, **100**, 551.



—— controls; - - - - tumor mice, total weight; —·— tumor mice minus tumors.

specific gravity, total nitrogen, protein, urea, allantoin, uric acid, creatine and creatinine, ammonia and amino acid nitrogen and the mineral constituents were determined. Only the first 4 of the above determinations showed significant differences (Figs. 1b, 1c, and 1d) for specific gravity, total nitrogen and protein, respectively. The changes in urea excretion paralleled those of the total nitrogen so closely that they were omitted from the figure.

The specific gravity (Fig. 1b) which in the normal mice varied from 1.065 to 1.057 in this series fell steadily after the first week in the tumor mice to 1.043 in the fourth week and rose somewhat before death.

That the above fall in concentration was due almost entirely to a diminution of urea and of the protein was shown by the decline in the total nitrogen, urea and protein but not in the other constituents, as shown in Figs. 1c and 1d for total nitrogen and protein respectively. During the first 3 weeks after tumor implantation the total urinary nitrogen did not vary from the normal level appreciably, but thereafter it fell abruptly, paralleling the fall in specific gravity. The urea nitrogen fell proportionately, but not the other nitrogenous constituents which remained unchanged.

A part of the decline in the total nitrogen excretion was evidently due to the gradual diminution of the protein content whose steady decrease to mere traces is shown in Fig. 1d.

The figures obtained for ammonia nitrogen and titratable acidity did not show any significant variation from the normal figures indicating the absence of any profound changes in the acid base equilibrium during the growth of the tumor.

It appears quite plausible from the above data that the formation and metabolism of the rapidly growing tumor in the organism of the mouse not only draws largely for its building and energy yielding materials on the body tissues but also attempts to conserve these by a diminished excretion of urea and of the urinary protein. It is attractive to speculate upon the origin and the fate of this chondromucoid-like protein. That it is constantly present in the urine of white mice indicates that it is a normal metabolic product in this animal species. As to its probable fate in the tumor bearing animals no conclusions can be reached at present. A study of the tumor proteins is being carried on with particular attention to the possibility of identifying a protein similar to that of the urine.

These observations are at variance with those of Kimura[3] in

[3] Kimura, Y., *J. Biochem.*, 1932, **8**, 469.

white rats with transplanted tumors of the Flexner sarcoma type. Kimura determined the nitrogenous and mineral constituents of the urine and reports an increase in the total nitrogen in the form of urea and allantoin paralleled by an increase in sulfate and phosphate excretion, indicating an increased rate of protein catabolism. Kimura was unable to find any protein and found only a trace of allantoin in normal rat urine, whereas in this laboratory protein has been found as a fairly constant constituent of rat urine and allantoin has been shown to be the predominant end product of purin metabolism in the rat by Hunter, Givens, and Guion.[4]

Summary. In the course of the growth of Sarcoma 180 in white mice the excretion of nitrogen diminishes chiefly at the expense of urea and of the urinary protein, with the latter tending to disappear entirely. The excretion of the other urinary constituents is practically unaffected.

6796

Formaldehyde Inhibition of Tryptic and Peptic Digestion of Egg White.*

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In connection with a study of the action of formaldehyde on certain bacterial suspensions,[1] parallel observations were made of its effect on trypsin and pepsin. Little work has been reported on the influence of formaldehyde on tryptic and peptic digestion of proteins. Johannessohn[2] found that preparations of trypsin vary in their susceptibility to this aldehyde; activity of one preparation was unaffected by 20 hours standing with 1% formaldehyde, while that of another was inhibited by 1/20% formaldehyde. He also reported that pepsin was not inactivated by 10% formaldehyde in 24 hours.

In our experiments, however, formaldehyde in low concentrations had a marked inhibitory effect on the action of both trypsin and pep-

[4] Hunter, A., Givens, M. H., and Guion, C. M., *J. Biol. Chem.*, 1914, **18**, 387.

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[1] Miller, C. P., Jr., and Boor, A. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 829.

[2] Johannessohn, Fritz, *Biochem. Z.*, 1917, **83**, 28.