Serum Polysaccharide Levels in Rats Bearing the Walker 256 Tumor*

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Previous work on human subjects (9) has shown that the polysaccharide associated with serum proteins is significantly elevated in malignancy. Little is known concerning the correlation between this elevation and the cancerous condition. Work in this laboratory has shown that the serum polysaccharide of dogs is increased in several types of experimental inflammation (7). An elevation also occurs in pregnancy (4, 10), tuberculosis, sarcoidosis, and other pathological conditions (6, 9). This communication deals with a study of serum polysaccharide during the progressive development of a rapidly growing transplantable rat tumor.

EXPERIMENTAL

Experimental animals.—Transplants of the Walker 256 carcinosarcoma1 were made subdermally on either the abdomen or the back, by means of a No. 15 hypodermic needle. Male rats of the Sprague-Dawley strain were used throughout. Age of the rats varied between 65 and 200 days of age at the time of transplantation. A successful transplantation rate of about 95 per cent was obtained. All analyses were made on pooled serum obtained by cardiac puncture from six rats in each group. A set of control animals was placed under the same environmental conditions and bled at the same time as each set of tumor animals. To provide some estimate of the rate of growth of the tumors, the width and breadth of the tumor were measured in millimeters each week. These measurements were then multiplied together; the results of this calculation are shown graphically in Figures 1 and 2.

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Chemical methods.—Serum was fractionated into albumin, albumin + \( \alpha \)-globulin, and \( \beta + \gamma \)-globulin fractions by the method of Milne (3) developed for human plasma, using 19.6 per cent and 26.0 per cent sodium sulfate to precipitate the globulin fractions. Nonglucosamine polysaccharide (referred to hereafter in this paper simply as polysaccharide) was determined as previously described (8) by the tryptophane method on the albumin and albumin + \( \alpha \)-globulin fractions after ethanolic precipitation (2 ml. of filtrate was added dropwise to 15 ml. of absolute ethanol). The polysaccharide associated with the \( \alpha \)-globulin fraction was obtained by difference between the two determinations and the polysaccharide associated with the combined \( \beta \) and \( \gamma \)-globulin by subtracting the polysaccharide of the albumin and \( \alpha \)-globulin fractions from the total serum polysaccharide. Protein was determined on all fractions by the biuret reaction.

RESULTS

Changes of protein distribution.—The average results of the polysaccharide and protein determinations are summarized in Tables 1 and 2. The results shown in Table 2 for normal rats agree fairly well with the electrophoretic data of Gjesing and Chanutin (1), who report 42-45 per cent albumin and 32-34 per cent \( \alpha \)-globulin in plasma protein from normal male rats of the Wistar stock. A decrease in serum albumin from 35 per cent to 26 per cent of the total serum proteins occurred in tumor-bearing rats after 5 weeks of tumor growth. Conversely, the \( \beta + \gamma \)-globulin fraction increased from 32 per cent to 41 per cent, while \( \alpha \)-globulin exhibited no change. During this period the control rats exhibited a small decrease in serum albumin and a slight increase in \( \alpha \)-globulin. In this connection it might be noted that Werner (11) noted a similar decrease in albumin and elevation of \( \alpha \)-globulin in rabbit sera following massive daily bleedings.

Some of the increase in total serum polysaccharide in the tumor-bearing rats is due to an increase of globulins relative to albumin. Thus, if all serum protein fractions of the tumor-bearing rats had the same polysaccharide content as those of the control rats, the total polysaccharide: total protein ratio 5 weeks after transplantation would be 3.38. The difference between this figure and the actual ratio (4.34) represents a change in the polysaccharide content of the serum fractions.

Changes of polysaccharide distribution.—The serum or serum fraction levels expressed by a figure obtained by dividing the polysaccharide level by the amount of protein in a given fraction are depicted graphically in Figures 1 and 2. These data were obtained by averaging results from four different groups of rats: two with transplants to the abdomen, one with transplants to the back, and one with transplants to both abdomen and back. No difference was noted among the groups. In a similar way, data for the controls were obtained by averaging results for the control groups carried simultaneously with each group of tumor-bearing animals.

The total nonglucosamine polysaccharide of the serum increased noticeably by the third week in all tumor-bearing groups. The protein polysaccharide of the albumin fraction was elevated noticeably after 2 weeks of tumor growth and became markedly elevated as the size of the tumor increased. This was found to be true for each group of rats as well as for the average of all groups. It is noteworthy that the increase in albumin polysaccharide roughly paralleled the figures obtained for the size of the tumor. In general, the polysaccharide content of the \( \alpha \)-globulin of the tumor-bearing rats was slightly elevated after the third week.
week, in comparison with that of the control group. However, in one group of rats this elevation was not found. When based on the average of all groups, the polysaccharide content of the combined \( \beta \)- and \( \gamma \)-globulin fraction rose in both tumor and control rats, reached a maximum, and then decreased; but this trend was marked in only one set of animals. It appears doubtful whether the polysaccharide content of the globulin fractions is connected in any direct way with the growth of the tumor.

DISCUSSION

The relatively small elevation of total serum polysaccharide in the normal rats during the course of the experiment was partly due to an increase in the serum \( \alpha \)-globulin of this group—possibly a response to the injury caused by the periodic bleeding. The chief cause of the elevation of serum polysaccharide in the tumor-bearing rats is attributed to the increased polysaccharide content of the albumin fraction. This finding is in agreement with those of Seibert, Pfaff, and Seibert (5), who were unable to completely account for the high serum polysaccharide content in human carcinoma patients by calculations based on the polysaccharide content of normal serum fractions and the distribution of these fractions in the sera of carcinoma patients, as determined by electrophoretic analyses. Apparently, there is an increase in some carbohydrate-rich fraction of serum which accompanies albumin during removal of the globulins by 26 per cent sodium sulfate. As results obtained by animal experimentation may not be directly applicable to human metabolism, similar fractionation studies are being conducted on sera from cancer patients. Investigations are also under way to determine what part of the polysaccharide associated with the albumin fraction is due to the mucoprotein fraction described by Winzler and Smyth (2, 12).

SUMMARY AND CONCLUSIONS

A study was made of the effect of tumor growth on the serum polysaccharide level of rats bearing the Walker 256 tumor. The total nonglucosamine polysaccharide increased greatly as the tumor size increased. Much of this increase was due to a polysaccharide associated with serum albumin as determined after the precipitation of globulins with 26 per cent sodium sulfate. The elevation occurred concurrently with an actual decrease in the albumin fraction relative to the globulin fraction in the sera of tumor-bearing animals. In the rat the polysaccharide content of the albumin fraction closely paralleled the tumor size, suggesting a possible relationship of this factor to tumor growth.

REFERENCES

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