Influence of Secondary Infection on Tumor Host Serum-Bound Carbohydrates. (28463)

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Numerous studies relating physiological and pathological factors to enhanced serum-bound carbohydrates have been reported. Serum glycoproteins of tumor-bearing hosts were shown to be elevated, but the mechanism of this phenomenon has not been explained. A number of hypotheses concerning the possible mechanism have been proposed (see review by Shetlar(1)). The possibility, however, that secondary factors, other than the tumor *per se,* may influence the serum-bound carbohydrates has not been eliminated. Recently, Kampschmidt *et al.*,(2,3) showed that certain systemic effects elicited in tumor-bearing animals were the result of bacterial-contaminated (*Salmonella typhimurium*) tumors and that the infection resisted antibiotics *in vivo*. In light of these studies, serum-bound carbohydrate levels in rats bearing "bacteria-free"* or "contaminated" tumors were followed, as well as extracts of these tissues.

Materials and methods. The "contaminated" and "bacteria-free" Walker 256 carcinosarcomas used for transplant were prepared by the procedures described by Kampschmidt *et al.*(2). The "bacteria-free" tumors were obtained by several passages through tissue culture in the presence of penicillin and streptomycin. The Novikoff hepatoma was obtained from Dr. Robert E. Greenfield, Nat. Inst. of Health. A primary hepatoma was induced by feeding 3'-methyl-4-dimethylaminoazobenzene for 8 weeks. All hosts were female Holtzman rats weighing 200-250 g. Bacterial contamination of tissues was determined by incubation of tissue slices in thioglycolate broth and observation of the turbidity for 24 hours at 37°C.

Tissue extracts were prepared according to the procedure described by Nakagawa *et al.*(4) and injected intraperitoneally as a suspension in 2 ml physiological saline (25 mg/animal). A lipopolysaccharide fraction of *S. typhimurium* (Difco Laboratories, Detroit, Mich.) was suspended in 2 ml physiological saline and injected at 100 μg levels per animal. Twenty-four hours after injection the animals were sacrificed.

Serum was obtained by cardiac puncture following a light anesthesia with sodium pentothal. Serum-bound hexose was determined by both the tryptophan method of Shetlar *et al.*(5) and the orcinol method of Winzler (6). No significant difference was noted between the 2 methods, and the values obtained by the orcinol procedure are reported. Sialic acid was determined by the direct method of Warren(7), hexosamine by the method of Winzler(6), and protein by the method of Lowry(8) with bovine albumin as standard.

Results. The serum-bound carbohydrate and protein levels of hosts bearing "contaminated" and "bacteria-free" tumors are shown in Table I. The carbohydrate values were elevated in hosts bearing the "contaminated" tumor, whereas the values were depressed or unaltered in hosts bearing tumors shown to be uncontaminated. The results obtained on reinfecting the tumor with *S. typhimurium* were similar to those for the "contaminated" tumor. Protein levels were depressed and the hexose-protein ratio elevated in all instances where the tumor was present, with the exception of the primary hepatoma-bearing host.

The effects of injecting tissue extracts and the lipopolysaccharide from the infecting organism are also included in Table I. A host response by enhancement of the serum-bound hexose was seen when the extract of "contaminated" tumor was injected. Similar results were obtained upon injection of the *S. typhimurium* lipopolysaccharide. No effects, however, were seen when extracts from the normal tissue or "bacteria-free" tumors were injected into the host.

To determine the relationship of "contaminated" and "bacteria-free" tumor growth to

* For the purposes of this paper, "bacteria-free" denotes lack of growth in thioglycolate broth.

TABLE I. Serum-Bound Carbohydrates of Hosts Bearing "Contaminated" and "Bacteria-Free" Tumors.

Host description	Infection*	Hexose, mg %	Protein, g %	Hexose/Protein ratio	Sialic acid, mg %	Hexosamine, mg %
Control	(—)	128.4 ± 2.7 (42)†	7.51 ±.16 (32)	1.79 ±.05 (28)	97.3 ± 3.4 (33)	144.8 ± 5.4 (19)
Tumors						
Walker 256	(—)	111.3 ± 4.4 (38)	5.58‡ ±.13 (26)	2.19‡ ±.07 (26)	108.8 ± 3.4 (23)	127.8 ± 6.4 (20)
Walker 256	(+)	153.4‡ ± 2.9 (49)	5.75‡ ±.19 (25)	2.73‡ ±.07 (23)	142.4‡ ± 2.5 (28)	166.2 ±10.3 (24)
Walker 256 (re-infected)	(+)	140.4 ± 9.1 (10)	6.03‡ ±.44 (11)	2.38‡ ±.14 (10)	126.1‡ ± 6.8 (5)	—
Novikoff I	(—)	118.5 ± 7.2 (30)	5.04‡ ±.09 (10)	2.54‡ ±.04 (10)	105.7 ± 4.0 (15)	—
Jensen	(—)	125.0 ± 4.6 (16)	5.18‡ ±.07 (9)	2.72‡ ±.05 (9)	91.5 ± 6.5 (16)	150.1 ± 4.3 (9)
Primary hepatoma	(—)	119.2 ± 2.7 (6)	7.04 ±.02 (6)	1.69 ±.04 (6)	—	—
Extracts§						
S. typhimurium	(+)	158.5‡ ± 3.4 (6)	6.59 ±.39 (6)	2.44‡ ±.13 (6)	—	—
Walker 256	(+)	155.8‡ ± 3.7 (5)	6.71 ±.14 (5)	2.33‡ ±.08 (5)	—	—
Walker 256	(—)	137.5 ± 3.1 (6)	6.74 ±.33 (6)	2.05 ±.09 (6)	—	—
Jensen	(—)	123.6 ± 3.8 (3)	7.28 ±.81 (3)	1.70 ±.04 (3)	—	—
Liver	(—)	132.6 ± 4.7 (3)	7.71 ±.23 (3)	1.72 ±.06 (3)	—	—

* Tumors "contaminated" with *S. typhimurium* = (+); "bacteria-free" tumors = (—).
† Standard deviation; No. in parentheses represent No. of animals.
‡ $P < .02$.
§ *S. typhimurium* lipopolysaccharide (100 μg) and tissue extracts (25 mg) were suspended in 2 ml physiological saline and injected intraperitoneally.

the altered serum-bound carbohydrates of the hosts, a time study was made using the "contaminated" (Fig. 1) and "bacteria-free" (Fig. 2) Walker 256 carcinoma. The average ratio of the values for the experimental animal to control animal was calculated using 3 animals per time period. Of interest was the extended lag period in the growth of the "contaminated" tumor (Fig. 1). At 7 days the "contaminated" tumors averaged only 0.2% of the body weight, while the "bacteria-free" tumors comprised 8.3% of the total body weight (Fig. 2). Further, the "bacteria-free" group had a shorter survival time.

In the "bacteria-free" group the bound hexose and hexosamine levels were unaltered at 3 days but decreased after the tumor had started its rapid phase of growth (Fig. 2). Protein values, on the other hand, had decreased by the 3rd day and remained at the same level for the remainder of the experiment. Sialic acid values were not altered throughout the period.

The bound carbohydrates of the "contaminated" hosts were elevated by the 3rd day, prior to any visible growth of the tumor, and remained elevated at the same level throughout the experiment. The serum protein level of the "contaminated" host continually decreased during tumor growth.

Discussion. The possibility that many of the protein abnormalities seen in patients with cancer might be attributed to the presence of secondary infection has recently been raised by Peterman(9). The findings of Kampschmidt *et al.*(2,3) that certain host effects heretofore attributed to tumor "toxohormone" were the result of a bacterial in-

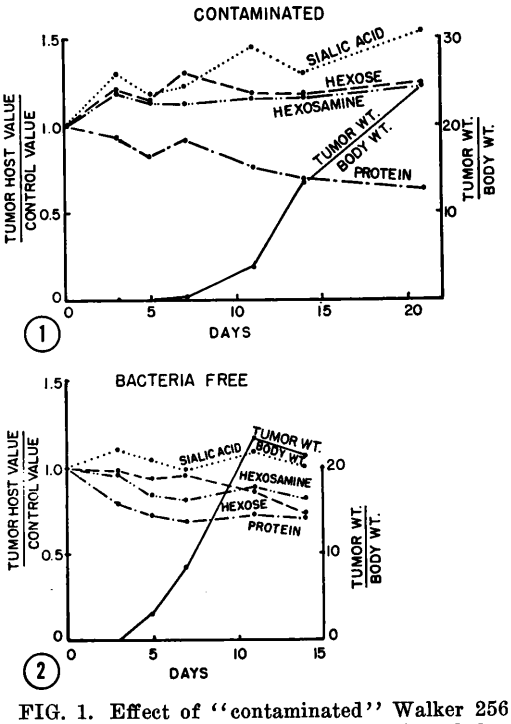

FIG. 1. Effect of "contaminated" Walker 256 carcinoma growth on host serum-bound carbohydrates.

FIG. 2. Effect of "bacteria-free" Walker 256 carcinoma growth on host serum-bound carbohydrates.

fection of the tumor, lends credibility to such an hypothesis. In the present study, depressed or unaltered levels of serum-bound carbohydrates were found in hosts bearing tumors shown to be free of bacterial infection. Hosts bearing "contaminated" tumors, however, showed elevated levels of serum-bound carbohydrates. Further, little if any correlation could be found between the period of alteration of the bound carbohydrate levels and "contaminated" tumor growth. The tumor effect on the bound carbohydrate level seen in the "bacteria-free" tumor host apparently is overshadowed by the response of the host to bacterial infection. It would appear, therefore, that "bacteria-free" tumors depressed some serum-bound carbohydrates, while bacterial infection of these tumors induced a host response of increased production of these components.

In view of the results obtained in these studies, a note of caution should be interjected in the interpretation of data using tumor-host systems. Infections of the tumor which are inaccessible to antibiotics administered in the conventional manner can overshadow or mask effects of the tumor of the host.

Summary. A comparison was made of serum-bound carbohydrates of hosts bearing Walker 256 carcinosarcoma contaminated with *Salmonella typhimurium* and "bacteria-free" tumor hosts. It was found that "contamination" resulted in increased levels of total serum-bound carbohydrates whereas the "bacteria-free" levels were decreased or unaltered. Relating the carbohydrate levels to tumor growth revealed that in the "contaminated" group the carbohydrate levels were elevated prior to the rapid phase of growth while the "bacteria-free" group showed decreased levels during this stage of growth. It was emphasized that caution should be exercised in evaluation of tumor-host data.

The authors wish to thank Dr. R. F. Kampschmidt and Mr. H. F. Upchurch for assistance in the early phases of this work and, also, Mr. Eugene Conway and Mr. Wilbur Whittle who performed the tissue culture.

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Received May 14, 1963. P.S.E.B.M., 1963, v113