The Action of Serum from Partially Hepatectomized Rats on Explants of Liver and Tumors*

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SUMMARY

Serum from rats after partial hepatectomy showed a stimulating action on liver explants. The magnitude of assimilation of inorganic phosphorus, which was added to the medium, was used as a measure of the phosphorylation activity. Very small metabolic changes could be determined by this method. The maximal stimulating action is found in serum obtained from blood samples drawn 48 hours after partial resection of the liver. The same serum produced striking inhibition of phosphorylation when added to the medium of solid tumor explants or to suspended ascitic tumor cells. Even permanent strains of tumor cultures were inhibited in their phosphorus incorporation by the addition of serum from partially hepatectomized animals. An inhibition of tumor growth by serum from partially hepatectomized rats had never been observed in vivo. This different action of serum from partially hepatectomized animals on liver and tumor seems to indicate essential metabolic differences between these tissues.

Blood serum of partially hepatectomized animals, obtained from blood drawn at certain intervals after hepatectomy, seems to stimulate liver metabolism (1–3, 7). Since the factor underlying this effect is elusive, some investigators have reported negative results (4, 5). The effect may, however, be reproduced at any time by the method which has been evolved. Even very small differences in metabolic rates can be gauged by using the intake of labeled compounds as a measure (8–10). The incorporation of radioactive phosphate in organic compounds is proportional to the intensity of phosphorylation processes.

MATERIALS AND METHODS

Two-thirds of the livers of adult, male, Sprague-Dawley rats weighing 160–180 gm. were resected. After 48 hours blood was obtained by heart puncture. The blood sample was kept at +4° C. for 15 minutes. Next, it was centrifuged for 15 minutes at 2000 × g. Blood from healthy control rats was obtained and treated in the same manner. The action of freshly obtained sera from normal and partially hepatectomized rats was tested on cultures of the following rat tissues: (a) normal adult liver, (b) embryonic liver (16th–20th day), (c) regenerating liver 48 hours after resection of two-thirds of the organ, (d) Walker carcinoma and Yoshida sarcoma, both in solid form (10–20 days after inoculation) and as ascites tumors (3–6 days after inoculation).

Inorganic radioactive phosphate (Na$_3$HP$^{32}$O$_4$, specific activity above 1 mc/µg P) is measurably taken up by the explants. Incorporation is inhibited by monoidic acetic acid and is absent in denaturated explants (8). Of the P$^{32}$ absorbed, an average of 44 per cent is found after 24 hr. of incubation in the fraction precipitated by trichloroacetic acid (12). The radioactive phosphate added to watch-glass cultures with a semi-solid medium amounts to 0.9 µc/ml, whereas in liquid media the final concentration is 0.2 µc/ml.

Liver and solid tumors were incubated in a fast-rotating roller apparatus in a liquid medium and in watch-glasses by the method of Fell and Robison. After sterile surgery the tissues were washed in ice-cooled Hanks solution and cut into cubic explants with a wet weight of ca. 1.5 mg. The small cubes were again washed in ice-cooled Hanks solution. Immediately after this, their wet weight was determined. Cylindrical tubes with a diameter of 10 mm. were filled with 0.5 ml. of Hanks solution and weighed. Next, eighteen ex-

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plant cubes were transferred to each tube, and the tube was weighed again. The error due to evaporation of liquid during the two readings was less than 1 per cent of the total weight. The explants inevitably drag along some liquid from the dish in which they are previously stored, and this is another source of error. However, this error was restricted to a minimum by the use of very small, thin knives. Since the same instruments were used in both the experimental and control series, the residual error was kept constant for either. Individual explants of absolutely equal weight can be made only with great difficulty, if at all; so we chose to use groups of eighteen small cubes as an experimental unit, hoping that inequalities would cancel out inside the group. This applied to both test and control cultures. The unit consisted of eighteen explants, weighed jointly, with a total wet weight of 27 mg. ± 5 per cent. Within this range, and given equal conditions, the intake of radioactive matter during the period of incubation is directly proportional to the wet weight of the explants (9).

Watch-glass technic.—Following the method of Fell and Robison, watch-glasses with a diameter of 20 mm. were placed in a moist chamber consisting of a Petri dish with a diameter of 50 mm. and covered with a semi-solid medium composed of 1 drop of chicken plasma, 1 drop of embryonic chick extract, 2 drops of Hanks salt solution, and 2 drops of rat serum. The serum was from partially hepatectomized rats in the test cultures and from normal untreated rats in the control cultures. All other conditions were kept equal for both series. When the medium had coagulated, three weighed cubic explants of liver or tumor tissue, prepared as described before, were placed on each watch-glass; therefore, one unit of eighteen explants required six watch-glasses. The cultures were incubated at 37° C. for 24 hr. Thereupon they were cut out of the semi-solid medium with small and thin knives. The eighteen explants of each unit were put together again and rinsed 3 times in Petri dishes with 10 ml. of Hanks solution. From this suspension the cells were transferred to each tube, and the explants were removed from the tubes, and the explants were rinsed 3 times with 10 ml. of Hanks solution. After the third rinsing the washing fluid was devoid of radioactivity. The radioactivity of every eighteen explants was stated in counts/mg wet weight. The error of this method is about 4 per cent (9).

Suspension cultures.—The action of serum from partially hepatectomized rats on ascitic tumor cells in suspension was studied, with the use of Yoshida ascites sarcoma (MY-RY strain) and Walker ascites carcinoma (MR-RM strain). Both tumors had been maintained in Sprague-Dawley rats and had been cytologically analyzed (14, 15). Ascitic tumors obtained from tumor-bearing animals were placed in twice the amount of ice-cold Hanks solution and centrifuged for 2 min. at 150 X g. The supernatant liquid was discarded, and the original volume was restored with fresh Hanks solution. This procedure was repeated twice. Finally the cells were stored in the original volume of Hanks solution. From this suspension equal volumes, equivalent to 3 X 10^8 cells, were taken for test and control experiments. Cultures were incubated at 37° C. in glass vessels, with a centrally arranged stirrer to prevent sedimentation of the cells. The medium for suspension cultures was composed of rat serum (13 per cent), from partially hepatectomized animals in the test and from normal animals in the controls), chick embryonic extract (7 per cent), and Hanks solution (80 per cent). The medium was, prior to the addition of serum, prepared in one vessel for both test and control, and radioactive phosphate was added. It was thus ascertained that the amounts of P^32 in either were equal. (This applies to all our experiments with liquid media.) Twenty-five ml. of medium were used for 3 X 10^6 tumor cells. Equal volumes of suspension were drawn at varying intervals from both tests and controls. The samples were centrifuged for 5 min. at 2000 X g. Aliquots of the supernatant were, after evaporation, used for determination of the radioactivity of the medium. The cellular residue was washed with 6
The effect is most obvious in explants of embryonic rat liver, obtained from embryos between the 15th and the 20th day of gravidity. For physiological reasons embryonic liver is inclined to grow in vitro, whereas adult liver shows little or no readiness to grow in culture. Correspondingly, we found a gradation in radioactivity absorbed: embryonic liver exhibited the largest intake, with regenerating liver coming next, and normal adult liver in the last place. Even with normal rat serum, regenerating liver showed an increase of 92 per cent in radioactive intake over normal liver in the same medium. In explants of regenerating liver, in a medium containing serum from partially hepatectomized rats obtained 48 hr. after surgery, we found an increase in radioactive intake amounting to 159 per cent, as compared with that of normal liver in normal serum (Table 1).

The addition of serum obtained 48 hr. after partial hepatectomy to liver tissue cultures had an effect contrary to that achieved by allowing such serum to act on solid tumor cultures (Table 2). Whereas liver explants showed an increase of 14–63 per cent in radioactivity absorbed, the intake of phosphate by tumor explants was markedly inhibited, all other conditions being equal (Table 2). Our experiments showed that phosphorylation in Walker carcinoma in vitro was inhibited to the extent of 38–65 per cent by the addition of serum from partially hepatectomized animals, whereas the degree of inhibition in Yoshida sarcoma was between 27 and 80 per cent. This inhibitory action of serum from partially hepatectomized rats has been confirmed by a very great number of experiments in our laboratory and is indeed even more obvious than the stimulating effect on liver explants.

Ascitic tumor cells of either type mentioned show a depression of the uptake of radioactivity. It is noticeable soon after the start of the experiment. As early as 4 hr. after the start, it is of the same order as the depression found in solid tumor explants after 4 hr. (Chart 1), whereas solid tumor explants fail to show after 4 hr. any significant difference in their behavior in normal serum and partial-hepatectomy serum.

As regards permanent strains in suspension cultures, HeLa cells exhibited the same depression (Table 3). L cells, however, showed no measurable deviation in their labeled phosphate metabolism when exposed to the action of serum from partially hepatectomized rats. Explants of organs other than liver are not acted upon by this partial-hepatectomy serum in any manner distinguishable from normal rat serum. In agreement with Ref. 13,
we found that kidney explants showed no difference in the action of either kind of serum.

DISCUSSION

The stimulating action of serum from partially hepatectomized rats, which has been known from experiments in vivo, has thus been demonstrated in vitro. This agrees with results achieved by other methods. Even in the system isolated in vitro, the organic incorporation of P³² is markedly increased. The assessment of phosphate intake is a highly sensitive criterion for changes in the intensity of phosphorylation in the tissues studied. Of the radioactive phosphate taken up, 44 per cent are found in the fraction precipitated by trichloroacetic acid. Absorption of phosphate can be reduced or inhibited by inhibition of oxidative and substrate phosphorylation (8). Conversely, stimulation of phosphorylation also increases phosphate intake. Serum from partially hepatectomized animals will produce, in suitable concentration, an average increase of 30 per cent in liver explants. The action is organ-specific but not species-specific (13). The factor contained in the serum is also found in the regenerating liver of the same animal (11).

The surprising inhibitory effect of partial-hepatectomy serum on phosphorylation in explants of transplantable tumors is of the order of 30—80 per cent. Solid tumor explants and suspensions of ascitic tumor cells are both inhibited by partial-hepatectomy serum. In suspension the effect comes earlier because mixing of cells with the medium is more intensive. Depression is recorded as soon as 45 min. after the addition of serum.

Suspension cultures of ascitic tumor cells are both inhibited by partial-hepatectomy serum. In suspension the effect comes earlier because mixing of cells with the medium is more intensive. Depression is recorded as soon as 45 min. after the addition of serum.

Suspension cultures of tumors maintained as permanent strains respond to partial-hepatectomy serum precisely as do solid tumor explants and cells freshly obtained from ascitic tumors. L cells do not respond at all. Surprisingly, the serum of...
rats with regenerating livers does not inhibit phosphate intake by cells of Ehrlich mouse ascitic tumor. Stasney, Paschkis, Cantarow, and Morris (6) found that the growth of tumors implanted in animals which had undergone partial hepatectomy was sometimes enhanced and sometimes inhibited, depending on the type of tumor used.

TABLE 3

<table>
<thead>
<tr>
<th>Cells</th>
<th>Addition to Culture Medium</th>
<th>Deviation II from I (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum from normal rats I</td>
<td>Serum from hepatectomized rats II</td>
</tr>
<tr>
<td>HeLa†</td>
<td>1604†</td>
<td>1446</td>
</tr>
<tr>
<td></td>
<td>1803</td>
<td>1478</td>
</tr>
<tr>
<td></td>
<td>1787</td>
<td>1398</td>
</tr>
<tr>
<td></td>
<td>1744 ± 55 §</td>
<td>1417 ± 79</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1708</td>
<td>1618</td>
</tr>
<tr>
<td></td>
<td>1699</td>
<td>1604</td>
</tr>
<tr>
<td></td>
<td>1423</td>
<td>1446</td>
</tr>
<tr>
<td></td>
<td>1610 ± 162</td>
<td>1566 ± 96</td>
</tr>
<tr>
<td></td>
<td>P&gt;0.1</td>
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</tbody>
</table>

* Obtained 48 hours after partial hepatectomy.
† Incubation time, 48 hours.
‡ Counts/min/total cells from one tube.
§ Mean values ± standard deviation.

We have incubated tumor cells before transplantation in low and high concentrations of partial-hepatectomy serum, but we have found no weakening of the virulence of such tumor cells. No effect results from the treatment of tumor-bearing animals with serum from partially hepatectomized rats.

The different action of serum from partially hepatectomized animals on liver and malignant tissue may be a sensitive indicator of a fundamental difference in metabolism, however small, in these two kinds of tissue.

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