Serum Tributyrinase Levels in Mice of the C57, C3H, and A Strains*

JULES TUBA

WITH THE TECHNICAL ASSISTANCE OF IRMGARD JESKE

(Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada)

A series of three papers by Khanolkar and Chitre (1, 3, 4) dealt with esterase studies in a cancer-resistant strain of mouse, C57, and in two cancer-susceptible strains, C3H and A. These authors showed that the hydrolysis of ethyl butyrate by mouse serum is twice as great in the cancer-susceptible animals as in the cancer-resistant group. There was no difference found in enzyme activity associated with the sex or age of the animals.

Studies in this laboratory on serum alkaline phosphatase (7), serum tributyrinase (10), and mammary cytochrome system of rats (8) have indicated that the sex and age of the animals may have significant effects on the levels of the enzymes. Our studies on the oxygen uptake of rat mammary tissue have demonstrated significant differences between breeder and virgin females in this regard. Unpublished findings with mice have shown us that age, sex, and previous pregnancy can affect the levels of certain enzymes. Consequently, it was decided to examine the effect of these factors on the serum tributyrinase concentrations of the strains of mice used by Khanolkar and Chitre. Our decision was strengthened by the fact that the simple titrimetric micromethod of Tuba and Hoare (9) was readily adaptable to investigations with mouse serum.

EXPERIMENTAL

Mice of various representative ages were housed in groups in metal cages and given Purina Fox Checkers and water ad libitum. Males and virgin females were segregated from the beginning of the experiment. Breeder females were segregated at least 4 weeks after the last litter was weaned, to eliminate any possible effect of lactation on the levels of the serum enzyme. There were 205 C3H, 104 C57, and 92 A mice used in the investigation.

For tributyrinase studies the animals were killed by decapitation, and blood was collected in 2-ml. conical centrifuge tubes. Serum was removed as soon as possible and stored at 4° C. until lipase determinations were done. This was never delayed for more than 3 days, although the enzyme was found to be stable for several days. For example, a sample of serum which showed an initial activity of 930 units was found to have an activity of 910 units/100 ml 4 days later, and this is within the limits of experimental error.

Micro-Kjeldahl tubes, which have bulbs of about 5 ml. in capacity, are used for the tributyrinase estimations. Into one of these tubes are pipetted with mixing: 0.05 ml. serum, 0.2 ml. water, and 1.0 ml. veronal buffer of pH 8.2. The mixture is warmed to 37° C., and then 0.02 ml. tributyrin, previously warmed to 37° C., is added with vigorous shaking of the contents of the experimental tube, in order to disperse the substrate. The contents of the tube are then agitated on a Warburg bath at the rate of 120 swings per minute, which is sufficient to maintain the substrate in a state of fine dispersion. It was found that under the above experimental conditions, which are optimal for studies of mouse tributyrinase activity, a hydrolytic period of 1 hour released sufficient butyric acid for accurate titration. Kinetic studies showed that enzyme action is proportional to time during this period and that activity varies directly with enzyme concentration for the amount of serum used.

After 1 hour, enzyme action in the experimental tube is terminated by the addition of 3 ml. of 95 per cent ethyl alcohol. The mixture is centrifuged, and the supernatant liquid is poured into a 50-ml. Erlenmeyer flask, which is immediately stoppered to prevent absorption of carbon dioxide. The contents of the flask are titrated with 0.025 N sodium hydroxide, with the use of phenolphthalein as an indicator. A control tube is used in which

* The mice for these investigations were made available through the kindness of Dr. H. E. Rawlinson, Department of Anatomy, University of Alberta.

The investigation was supported by a grant from the National Cancer Institute of Canada.

Received for publication October 1, 1951.
the serum is boiled before the addition of buffer and substrate.

The difference between titration values for the experimental and control tubes is a measure of the activity of the enzyme. Tributyrinase activity of mouse serum in units is equivalent to the number of ml. of 0.025 N sodium hydroxide required to neutralize the amount of butyric acid which is set free by the enzyme contained in 100 ml. serum under the above conditions. In replicate determinations the average deviation from the mean value was 2–3 per cent.

RESULTS

Each of the three strains was divided into a group of young animals (12–20 weeks), a middle-aged group (30–40 weeks), and an old group (50–60 weeks). The animals were also grouped as males, breeder females, and virgin females. In Table 1 the ranges of tributyrinase levels and means are given for each group of mice. The standard errors of the means are given for the total males, breeders, and virgins of each strain, and they are, in every case, less than 5 per cent of the mean value.

The statistical analysis of the tributyrinase means given in Table 1 is presented in Table 2. The data for the various groups have been compared for differences which might be due to age, sex, or strain. Those comparisons which show statistically significant differences are included in the table. In each case the t value is given, and levels of significance are indicated by values of P. Probability of 0.05 or less is significant, while a probability of 0.01 is highly significant.

DISCUSSION

It can be seen in Table 2 that age has a significant effect on serum tributyrinase levels in some groups. However, it cannot be said that any definite age pattern exists which may be associated with susceptibility or resistance to mammary carcinoma. Breeder females of the three strains show an age effect, which in the case of the C3H group is the reverse of the trend observed in the other two strains. Age effects are found with males and virgins, but these are not common to the three strains.

The effect of sex on serum tributyrinase levels, like that of age, is most pronounced in C3H mice, and males, breeders, and virgins of this strain are all significantly different in one or another of the age groups. Male-breeder differences and male-virgin differences exist in all three strains. Breeder-virgin differences are significant in C3H and C57 mice and are just beyond the range of significance in the A mice. The trend in the A strain would probably be more definite with a larger group of animals. Hormonal influences associated with the sex and previous pregnancy are therefore factors in determining the serum tributyrinase levels of the three strains of mice used in the investigation.

There are highly significant strain differences among the breeders and also among the males. The virgins of the three strains show no significant difference in enzyme level. We find, as do Khanolkar and Chitre with their esterase, that mice of the three strains, undifferentiated as to age, sex, and previous pregnancy, have significantly different levels of serum tributyrinase. The mean values, with standard errors of the means, for C57, A, and C3H mice, respectively, are 932 ± 12, 1045 ± 18, and 900 ± 8 units/100 ml of serum tributyrinase. There is no relationship between these levels and the degree of mammary cancer incidence; in fact, the mean for the C3H strain is less than that for the C57 animals. Shimkin, Greenstein, and Andervont (6) found that there is no correlation between the degree of hydrolysis of methyl butyrate by mouse serum and the occurrence of murine mammary tumors.

There are a number of factors which could account for the points of disagreement which exist between our results and those of Khanolkar and Chitre. The number of animals used has, of course, an important bearing on the statistical evaluation of results. It was noted above that the breeder-virgin difference in the A strain is just below the level of significance, probably because the numbers of animals are smaller than in the other groups. Variations in technic undoubtedly account for some of the differences between the two sets of results. It is our experience that the use of boiled serum in the control tube gives a more accurate measurement of the amount of hydrolysis which occurs in the experimental tube, since the buffering capacities of various sera are not always the same. A shorter hydrolytic period is usually preferable. Since the substrate was apparently not emulsified in the esterase determinations of Khanolkar and Chitre, it is possible that saturation of the enzyme was not always achieved.

We have estimated the extent of hydrolysis of ethyl butyrate by the sera of four 20-week-old male mice of each of the three strains. The method of Tuba and Hoare (9) employs veronal buffers, and, by the use of their technic, optimum hydrolysis was obtained at pH 7.8. The values for 20-week-old male C3H, C57, and A mice are respectively 390 ± 10, 417 ± 12, and 387 ± 18. These results, like those of Shimkin et al. (6),
### TABLE 1
**SERUM TRIBUTYRINASE LEVELS**

<table>
<thead>
<tr>
<th>Males</th>
<th>B&gt;R E D E R</th>
<th>F E M A L E S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>No. of mice</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tributyrinase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-20</td>
<td>8</td>
<td>740-950</td>
</tr>
<tr>
<td>30-40</td>
<td>38</td>
<td>710-1,000</td>
</tr>
<tr>
<td>50-60</td>
<td>93</td>
<td>900-1,150</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>710-1,150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Virgins</th>
<th>Tributyrinase</th>
<th>(units/100 ml)</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-20</td>
<td>18</td>
<td>750-1,040</td>
<td>935</td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>20</td>
<td>710-1,040</td>
<td>938</td>
<td></td>
</tr>
<tr>
<td>50-60</td>
<td>4</td>
<td>750-950</td>
<td>907</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>710-1,040</td>
<td>889 ±15*</td>
<td></td>
</tr>
</tbody>
</table>

*Standard error of the mean.

### TABLE 2
**STATISTICAL ANALYSIS OF DATA OF TABLE 1**

#### Age Differences

<table>
<thead>
<tr>
<th>Strain</th>
<th>Groups compared</th>
<th>Value of t</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>19-20-week males vs. 50-60-week males</td>
<td>3.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>50-60-week breeders vs. 50-60-week breeders</td>
<td>5.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>19-20-week virgins vs. 50-60-week virgins</td>
<td>2.53</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>A</td>
<td>30-40-week breeders vs. 50-60-week breeders</td>
<td>3.86</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57</td>
<td>30-40-week breeders vs. 50-60-week breeders</td>
<td>3.88</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57</td>
<td>19-20-week virgins vs. 50-60-week virgins</td>
<td>2.04</td>
<td>0.01-0.02</td>
</tr>
</tbody>
</table>

#### Sex Differences

<table>
<thead>
<tr>
<th>Strain</th>
<th>Groups compared</th>
<th>Value of t</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH</td>
<td>30-40-week males vs. 50-60-week males</td>
<td>6.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>50-60-week males vs. 50-60-week males</td>
<td>3.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>19-20-week males vs. 19-20-week virgins</td>
<td>3.84</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>CSH</td>
<td>50-60-week males vs. 50-60-week males</td>
<td>3.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>All males vs. all breeders</td>
<td>7.84</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSH</td>
<td>All breeders vs. all virgins</td>
<td>7.43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A</td>
<td>50-60-week males vs. 50-60-week virgins</td>
<td>2.79</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>A</td>
<td>All males vs. all virgins</td>
<td>2.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57</td>
<td>50-60-week breeders vs. 50-60-week virgins</td>
<td>2.68</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>C57</td>
<td>All males vs. all breeders</td>
<td>2.70</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57</td>
<td>All males vs. all virgins</td>
<td>2.08</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

#### Strain Differences

<table>
<thead>
<tr>
<th>Strain</th>
<th>Groups compared</th>
<th>Value of t</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 and CSH</td>
<td>Total breeders vs. total breeders</td>
<td>4.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57 and A</td>
<td>Total breeders vs. total breeders</td>
<td>3.19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57 and CSH</td>
<td>Total males vs. total males</td>
<td>4.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57 and A</td>
<td>Total males vs. total males</td>
<td>3.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C57 and CSH</td>
<td>Total mice vs. total mice</td>
<td>2.19</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>C57 and A</td>
<td>Total mice vs. total mice</td>
<td>5.06</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
show no correlation between enzyme activity and susceptibility to cancer.

Our findings are negative in the sense that we find no distinction in serum tributyrinase levels which can be associated with the incidence of murine mammary cancer. On the other hand, the results are in keeping with our observations of other enzymes in rats and mice: that is, age, sex, and previous pregnancy are factors which may affect enzyme activity and which should be taken into account. Morrow, Carroll, and Greenspan (5) have found that sex and age are factors in determining kidney glucuronidase levels in inbred mice. Harris and Cohen (2) report a pronounced influence of ovarian hormones on the activity of various enzymes, including esterase, in the tissues of Wistar strain female mice.

SUMMARY

1. Serum tributyrinase levels have been determined for mice of the C3H, A, and C57 strains.
2. Statistically significant effects on enzyme activity are associated with age, sex, previous pregnancy, and strain.
3. There is no evidence that the serum tributyrinase activity is related to susceptibility or resistance to cancer.

REFERENCES

4. ———. Studies in Esterase (Butyric) Activity. III. The Effect of Foster Nursing on the Esterase Content of Blood Serum and Liver of Mice Susceptible or Insusceptible to Mammary Cancer. Ibid., 4:189-33, 1944.
Serum Tributyrinase Levels in Mice of the C57, C3H, and A Strains

Jules Tuba and Irmgaard Jeske


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/12/2/113

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.