STUDIES ON ENZYME ACTION.

XXXV. LIPASE ACTIONS OF EXTRACTS OF TISSUES OF RABBITS AT DIFFERENT AGES.

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INTRODUCTION.

In a study of the lipase actions on ten different esters, of aqueous extracts of whole rats whose ages ranged from 3 days before birth to 3 years 15 days, it was found that the "pictures" of the relative actions on the different substrates changed progressively with increasing age of the rat. For the embryo and the youngest rats, the curves approached those given by the Flexner-Jobling rat carcinoma and by a number of tumors of human origin, changing to a type characteristic of the adult rat, and appearing to revert again to some extent toward the embryonic type for the oldest rats. The absolute actions on the different esters were found to increase and then to decrease again as the rats became older, the extent of the changes differing with the various esters.

As it was desired to study the changes in these enzyme actions of individual tissues with increase in age of the animal, and as the young rat is too small to use for this purpose, at the suggestion of Dr. C. B. Davenport the rabbit was chosen for this investigation. A study of a

2 Some early studies showed that the lipase activity of rabbit, pig, and sheep embryos increased with age (Buxton, B. H., and Shaffer, P., J. Med. Research, 1904–05, xiii, 549), and that the actions on ethyl butyrate of the liver and intestines of the adult pig were greater than those of the embryo (Mendel, L. B., and Leav- worth, C. S., Am. J. Physiol., 1908, xxi, 95).
number of tissues of the adult rabbit indicated differences in enzyme relations which should be of value in the study of similar properties with rabbits of different ages.

In this paper, results will be presented for the lipase actions of the following tissues of the rabbit: lung, liver, kidney, leg muscle, heart muscle, skin, spleen, stomach, small intestine, and brain. The ages of the rabbits ranged from 8 days before birth to 5 years 11 months 2 days.

Experimental Methods.

Rabbits of various breeds were used. Several of them were obtained from the Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, while the six oldest were obtained from the Bussey Institution, Harvard University. For these the authors desire to thank Dr. C. B. Davenport and Dr. W. E. Castle. The ages of those which were obtained before birth were calculated on the assumption of a gestation period of 30 days. The very young rabbits were killed with ether, the older by a blow below the atlas. The tissues were removed immediately, passed through a meat chopper, or ground with sand in a mortar, taken up with water, toluene added, extracted at room temperature overnight, filtered through paper, cloudy liquids being obtained as a rule, and brought to pH 7.0. The conditions of testing the lipase actions were the same as those described previously; 15 cc. of solution, 3.4 milli-equivalents of each of the ten esters, 22 hours incubation at 37–38°C, titration with 0.1 normal sodium hydroxide solution with phenolphthalein as indicator, duplicate and blank determinations, toluene present throughout.

Experimental Results.

The experimental results will be given only for the tissues of rabbits whose ages are definitely known. As in previous papers, two methods of comparing the enzyme actions present themselves; either as relative

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The relative actions given in the chart show a regular progression with increase in age of the rabbits. For the youngest, the type is very much like that found with the whole rat embryo, with the Flexner-Jobling rat carcinoma, and with a number of human tumors. As a matter of convenience, it may for the present be referred to as the embryonic type. A change in the type apparently begins at the age of 14 days and may be said to be complete at 195 days. The type then remains fairly constant except that with the oldest rabbits, a reversion toward the original younger type is appearing with the acetates. It is unnecessary to go into details with the individual esters, as the changes are very apparent. Some irregularities appear, at times with individual esters, very occasionally with a group of esters with one rabbit. Such irregularities are unavoidable, and in fact to be expected, in dealing with experimental data from separate biological units.
actions of a given extract on a number of esters, or as absolute actions. The results for any one tissue will be given in the form of curves for the relative actions and tables for the absolute actions in as much detail as seems advisable in each case to bring out the various relations.

TABLE I.

Hydrolyzing Actions in Tenths of Milli-Equivalents of Acid Produced by Kidney Extracts of Rabbits of Different Ages on the Indicated Esters.

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<td>1.58</td>
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<td>0.94</td>
<td>0.74</td>
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</table>

In the curves for showing the relative actions the esters are arranged along the abscissa axis in the same order as in the previous papers. As a rule, four curves are given on each plot; in some cases which will be indicated, three, five, or six are shown.
In the discussion of the results, the different physiological periods (epochs) of the life of the rabbit may be recalled; i.e., embryonic, nursing and weaning, young, adult. The embryonic is quite uniformly 30 days. The nursing rabbit begins to eat food other than milk at about the age of 2 weeks, and, as a rule, is weaned at about 6 weeks. The onset of puberty is quite variable. In general, rabbits become sexually mature between 5 and 7 months of age.

The consideration of the absolute actions on the different esters, of the kidney extracts of the rabbits of different ages is essential for a satisfactory understanding of the changes in these enzyme actions. These values are given in Table I.

The results for the five youngest rabbits were obtained with extracts corresponding to 8.9 mg. original tissue per cc. final solution tested, the remaining results refer to double this concentration. The striking fact for these absolute actions is their large increase with increasing age, up to a certain point. For the older rabbits definite decreasing actions are observed with benzyl, ethyl, methyl, isobutyl acetates, methyl and ethyl benzoates; possible decreases with phenyl acetate and glycercyl triacetate, but no decreases with methyl and ethyl butyrates. The absolute actions for the 1221 days old rabbit are considerably larger than those for the rabbits somewhat younger and somewhat older. The relative actions, however, fall in line with those of the other rabbits. The absolute actions found with the other tissues of this rabbit are also greater than would be expected from the results with the other rabbits, but this apparently exceptional behavior is not reflected in the types or pictures of the relative actions which correspond to the results obtained with the other rabbits. These results can therefore only be recorded and no explanation suggested for them at present. It is also apparent that the very marked increases in the enzyme actions begin after the age of 14 days.

The physiological behavior of the rabbit kidney may be considered briefly in this connection. The embryonic kidney practically does not function. While the rabbit is on a milk diet only (up to the age of 10 days or so) the kidney activity is at a comparatively low level. After that the milk is supplemented by other foods and the kidney functions more actively. It is interesting that the change in enzyme action (both relative and absolute) becomes striking at this period. There
FIG. 2. Liver Extracts. These pictures or types of actions show considerable similarity throughout the life cycle of the rabbit. At one period, however, there is a definite difference. Between the ages of $2\frac{1}{2}$ and 28 days there is a partial reversion in the direction of the embryonic type. The relative actions of the butyrates are markedly less here. Between the ages of 7 and 35 days, the actions on benzyl, ethyl and methyl acetates are much the same, thereafter the values increase in the order listed. The adult type may be said to be present after the age of 45 days. For the oldest rabbits, there are indications of a reversion as shown by the decrease in the three acetate ratios mentioned and by the decrease in the actions on the benzoates.
is also a possible correlation of physiological activity with the decrease in absolute lipase actions (small, but apparent) noted after puberty (about 200 days). The metabolism per kilogram is highest in the young. It decreases gradually until puberty when the curve flattens out and the rate of decrease with increasing age is very slow. The metabolism is then lower per kilogram or per square meter of surface, but the total amount of substances excreted by the kidneys would probably not be much, if any, less.

The absolute actions of these liver extracts are shown in Table II. The results refer to the content of 17.8 mg. original solid per cc. of final solution tested.

### Table II

**Hydrolyzing Actions in Tenths of Milli-Equivalents of Acid Produced by Liver Extracts of Rabbits of Different Ages on the Indicated Esters.**

<table>
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<th>Age (days)</th>
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<th>MIOAc</th>
<th>PISIOAc</th>
<th>EIOAc</th>
<th>MIOAc</th>
<th>EISIOAc</th>
<th>MIOAc</th>
<th>EISIOAc</th>
<th>MIOAc</th>
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</table>
Fig. 3. Lung Extracts. The types of action for the three youngest rabbits approach the embryonic type. At birth or immediately thereafter, the type changes, the change being fairly complete at 14 to 21 days. In reaching this adult type, the actions on all the esters relative to phenyl acetate increase except that on glycercyl triacetate which decreases, and that on isobutyl acetate which does not change. For the oldest rabbits a certain amount of reversion is apparent. This can be seen in following the complete life cycle with the butyrates and with the benzoates which increase and finally decrease again. On the other hand the ratios of the actions on the butyrates to those on glycercyl triacetate increase from the embryo to the adult and remain constant and for the actions on benzyl acetate to those on ethyl benzoate (its isomer) decrease and then remain constant. Additional regularities involving various pairs of esters might be indicated but will not be given because of limitation of space.
In general terms these actions increase with increasing age of the rabbits. During the nursing period before the milk is supplemented by other foods, the first 2 weeks of life, the butyrates show a marked drop. All the esters show increases beginning at about 28 to 35 days,

TABLE III.

<table>
<thead>
<tr>
<th>Age</th>
<th>DICAc</th>
<th>GICAc</th>
<th>MoCOAc</th>
<th>PrHCOAc</th>
<th>EICAc</th>
<th>MoAc</th>
<th>EICOAc</th>
<th>MoBS</th>
<th>BoAc</th>
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<td></td>
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<td></td>
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<tr>
<td>-8 (dil.)</td>
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<td>0.50</td>
<td>0.32</td>
<td>0.18</td>
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<td>0.16</td>
<td>0.30</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>-7 (dil.)</td>
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<td>0.44</td>
<td>0.40</td>
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<td>0.62</td>
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</tr>
<tr>
<td>0 (dil.)</td>
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<td>0.41</td>
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<td>0.98</td>
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<td>0.82</td>
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<td>0.80</td>
<td>0.86</td>
<td>0.90</td>
<td>1.80</td>
<td>0.72</td>
<td>0.62</td>
</tr>
<tr>
<td>14½</td>
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<td>1.36</td>
<td>2.08</td>
<td>0.82</td>
<td>0.82</td>
<td>1.00</td>
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<td>0.86</td>
<td>0.84</td>
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<tr>
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<td>1.49</td>
<td>2.10</td>
<td>0.59</td>
<td>0.82</td>
<td>0.86</td>
<td>1.90</td>
<td>0.64</td>
<td>0.54</td>
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<tr>
<td>35</td>
<td>3.19</td>
<td>1.72</td>
<td>2.96</td>
<td>0.94</td>
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<td>1.44</td>
<td>2.51</td>
<td>0.84</td>
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<td>1.22</td>
<td>2.15</td>
<td>0.80</td>
<td>0.88</td>
<td>0.98</td>
<td>1.90</td>
<td>0.68</td>
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<td>0.68</td>
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<tr>
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<td>2.31</td>
<td>0.70</td>
<td>0.99</td>
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<td>1.22</td>
<td>2.08</td>
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<td>1.02</td>
<td>1.41</td>
<td>1.70</td>
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<td>2.25</td>
<td>0.72</td>
<td>0.92</td>
<td>0.97</td>
<td>1.88</td>
<td>0.67</td>
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<td>2.79</td>
<td>0.78</td>
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<td>1.34</td>
<td>2.28</td>
<td>0.73</td>
<td>0.61</td>
</tr>
<tr>
<td>1737</td>
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<td>1.71</td>
<td>3.49</td>
<td>1.06</td>
<td>1.05</td>
<td>1.20</td>
<td>2.77</td>
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<td>0.64</td>
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<tr>
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<td>3.49</td>
<td>1.03</td>
<td>1.20</td>
<td>1.38</td>
<td>2.74</td>
<td>0.88</td>
<td>0.94</td>
</tr>
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</table>

these changes after weaning are especially large with the normal alkyl esters, methyl and ethyl butyrates and acetates.

With reference to the physiological changes, it may be stated that the liver may, and probably does, function during late embryonic life. During the nursing period the diet is restricted to an ideal food. There is then less "detoxication" by the liver—formation of conjugate
FIG. 4. Skin Extracts. Curves for three ages are shown in the first five plots, four on the sixth. No skins of embryo rabbits were studied. Comparing the types of the skins of adult rabbits, which are markedly constant, with those of the younger rabbits, a difference is seen in that the results for the younger approach to a certain extent the embryo type of enzyme action, and which consequently suggest that with skin of embryos, the results would show completely the embryonic picture for the enzyme actions. This is brought out in the increasing relative actions of the butyrates and the benzoates. The actions on benzy1, ethyl, and methyl acetates,—much the same for the younger rabbits, increasing in the stated order of the esters for the older,—are of interest.
H. M. NOYES, K. G. FALK, AND E. J. BAUMLANN

acids such as glycuronic acid, etc.—in which the liver is supposed to play a part. This may account for the reversion observed in the enzyme actions, both relative and absolute, during the nursing period.

The absolute actions of the lung extracts are shown in Table III.

The lung extracts of the rabbits aged $-8$, $-7$, $0$, and $\frac{1}{2}$ days refer to concentrations of 8.9 mg. tissue per cc. of solution tested, the remaining extracts were made up with 17.8 mg. per cc.

**TABLE IV.**

*Hydrolyzing Actions in Tenths of Milli-Equivalents of Acid Produced by Skin Extracts of Rabbits of Different Ages on the Indicated Esters.*

<table>
<thead>
<tr>
<th>Age, days</th>
<th>PmOAc</th>
<th>GluAc</th>
<th>McOAc</th>
<th>PmHAc</th>
<th>GluAc</th>
<th>McOAc</th>
<th>McOAc</th>
<th>McAc</th>
<th>McOAc</th>
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<td>0.41</td>
<td>0.21</td>
<td>0.15</td>
<td>0.14</td>
<td>0.34</td>
<td>0.02</td>
<td>0.00</td>
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<tr>
<td>1</td>
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<td>0.42</td>
<td>0.22</td>
<td>0.15</td>
<td>0.20</td>
<td>0.34</td>
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<tr>
<td>$6\frac{1}{4}$</td>
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<td>0.56</td>
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<td>0.20</td>
<td>0.21</td>
<td>0.35</td>
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<td>0.25</td>
<td>0.43</td>
<td>0.08</td>
<td>0.06</td>
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<td>0.63</td>
<td>0.13</td>
<td>0.14</td>
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<td>0.51</td>
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<td>0.63</td>
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<tr>
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<td>0.59</td>
<td>0.36</td>
<td>0.72</td>
<td>0.17</td>
<td>0.15</td>
<td>0.25</td>
<td>0.60</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>45</td>
<td>0.72</td>
<td>0.50</td>
<td>0.90</td>
<td>0.30</td>
<td>0.29</td>
<td>0.37</td>
<td>0.81</td>
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<td>0.18</td>
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<td>0.82</td>
<td>0.53</td>
<td>0.74</td>
<td>0.29</td>
<td>0.24</td>
<td>0.26</td>
<td>0.61</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>102</td>
<td>0.84</td>
<td>0.51</td>
<td>1.00</td>
<td>0.18</td>
<td>0.26</td>
<td>0.38</td>
<td>0.87</td>
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<td>0.16</td>
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<td>0.36</td>
<td>0.75</td>
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</tr>
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<td>0.12</td>
<td>0.24</td>
<td>0.41</td>
<td>0.98</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
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<td>0.41</td>
<td>0.86</td>
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<td>0.31</td>
<td>0.76</td>
<td>0.20</td>
<td>0.14</td>
</tr>
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</table>

There is evidently an increase in the absolute enzyme action at or in the close neighborhood of birth. The increases on the various esters continue up to apparent maxima whose exact points or times are difficult to determine because of the irregularities in the individual cases but which appear to occur at about 14 days. No real decreases in the absolute actions can be stated to occur with the oldest rabbits.
Fig. 5. Leg Muscle Extracts. As shown in the following table (Table V), eight of these series were obtained at a concentration of 17.8 mg. tissue per cc. of solution tested, one at 35.6 mg., fourteen at 44.4, and one (102 days) the average of the relative actions obtained at 17.8 mg. and 44.4 mg. The absolute actions were comparatively small at the lower concentration. This fact would increase the percentage error in the relative actions, and would account for the greater irregularities found with the curves in this figure. Even under these conditions, definite trends in the types or pictures may be observed. At the earliest ages studied, the types were considerably different from the embryo type although two of these curves (−3 days and 0 days) in the first group are somewhat similar to it. In the second group (½ to 14½ days) there is a real return to the embryo type. The drop in the actions on the butyrates and benzoates and the increase in glyceryl triacetate is unquestioned. Thereafter there is a development of the adult type which is apparently complete after about 3 months.
although some of the minor changes which take place appear to be reflected in small changes in the relative actions especially when pairs of esters only are considered, as pointed out in the discussion of the relative actions of these extracts.

### TABLE V.

**Hydrolyzing Actions in Tenths of Milli-Equivalents of Acid Produced by Leg Muscle Extracts of Rabbits of Different Ages on the Indicated Esters.**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>PA0Ac</th>
<th>GI0Ac</th>
<th>MO-COP</th>
<th>PAC0Ac</th>
<th>EtOAc</th>
<th>MOAc</th>
<th>EN0Ac</th>
<th>MOBa</th>
<th>EIOBa</th>
<th>i-Et0Ac</th>
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<td>0.71</td>
<td>0.57</td>
<td>0.56</td>
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<td>0.18</td>
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<td>0.46</td>
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<td>0.04</td>
<td>0.34</td>
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<tr>
<td>7 (17.8)</td>
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<td>0.52</td>
<td>0.65</td>
<td>0.27</td>
<td>0.21</td>
<td>0.26</td>
<td>0.59</td>
<td>0.15</td>
<td>0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>5 (17.8)</td>
<td>0.79</td>
<td>0.60</td>
<td>0.42</td>
<td>0.24</td>
<td>0.16</td>
<td>0.19</td>
<td>0.29</td>
<td>0.02</td>
<td>0.03</td>
<td>0.24</td>
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<tr>
<td>0 (17.8)</td>
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<td>0.48</td>
<td>0.32</td>
<td>0.18</td>
<td>0.22</td>
<td>0.33</td>
<td>0.04</td>
<td>0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>½ (17.8)</td>
<td>0.54</td>
<td>0.40</td>
<td>0.20</td>
<td>0.16</td>
<td>0.09</td>
<td>0.14</td>
<td>0.16</td>
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<td>0.00</td>
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</tr>
<tr>
<td>2½(17.8)</td>
<td>0.74</td>
<td>0.49</td>
<td>0.28</td>
<td>0.20</td>
<td>0.14</td>
<td>0.17</td>
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<td>0.00</td>
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</tr>
<tr>
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<td>0.15</td>
<td>0.10</td>
<td>0.18</td>
<td>0.24</td>
<td>0.00</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>195 (17.8)</td>
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<td>0.74</td>
<td>0.15</td>
<td>0.12</td>
<td>0.21</td>
<td>0.52</td>
<td>0.15</td>
<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>842 (17.8)</td>
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<td>0.38</td>
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<td>1.12</td>
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<td>0.36</td>
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<td>0.86</td>
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In considering the physiological relations in connection with these enzyme actions, the striking fact is the great change which occurs at birth when breathing begins.

The absolute actions of the skin extracts are shown in Table IV.
The changes in the absolute actions with increase in the ages of the rabbits are small compared to those found with some of the other tis-

![Graphs showing enzyme action changes with age.](image)

**Fig. 6. Heart Muscle Extracts.** The pictures for the youngest rabbits, as far as can be told from the few results obtained because of the small amount of material available, approach the embryonic type, diverging steadily as the rabbits become older until the adult type is reached at about 6 months. The relative increases in the butyrates and benzoates and decrease in glyceryl triacetate are noteworthy. There appears to be a real reversion for the oldest rabbits. Because of the comparatively small absolute actions, the percentage errors in the relative actions are magnified, and the resulting curves somewhat irregular.
The results given refer to the concentration of 17.8 mg. skin per cc. of solution tested. The values for the acetates remained practically unchanged except that the glyceryl triacetates decreased after 4 months. On the other hand, the values for the butyrates and benzoates increased markedly for the first few months and then remained constant. In general, the actions on the methyl esters were greater than on the corresponding ethyl esters.

<table>
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<tr>
<th>Age</th>
<th>PbOAc</th>
<th>GlOAc</th>
<th>MoOAc</th>
<th>PbC14OAc</th>
<th>BrOAc</th>
<th>MoOBr</th>
<th>BrC14OAc</th>
<th>MoOBz</th>
<th>BrOBz</th>
<th>PbOBz</th>
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</tr>
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<tr>
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<td>0.24</td>
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<td>0.00</td>
<td>0.40</td>
</tr>
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<tr>
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<td>0.35</td>
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<td>0.73</td>
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<td>0.18</td>
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<td>0.26</td>
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<tr>
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<td>0.37</td>
<td>0.25</td>
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<tr>
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<td>0.62</td>
<td>0.22</td>
<td>0.18</td>
<td>0.20</td>
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</table>

The absolute actions of the leg muscle extracts are shown in Table V.

The concentrations of the extracts in terms of mg. of tissue extracted per cc. of solution tested are given in parentheses in Column 1 following the ages of the rabbits. The decrease in the actions on the butyrates and benzoates just before birth and during lactation, followed by the
increase after weaning were referred to in speaking of the relative actions. Beyond this the variations are irregular and point in no definite direction.

The absolute actions of the heart muscle extracts are shown in Table VI.

![Graphs of enzyme action](image)

**Fig. 7. Brain Extracts.** The relative actions are shown, six on the first plot, five on each of the other three plots. There is practically no change in the type throughout the life cycle. There is a small apparent increase in the relative actions of the butyrates at the ages between 45 and 136 days but in view of the comparatively small absolute actions of the extracts, as shown in Table VII, this increase is not at all certain and may be due to the experimental inaccuracies.

The type of action, is in general, similar to the embryo type. Minor differences may be observed in the smaller actions of the brain extracts on the esters following phenyl acetate and glycercyl triacetate, in the actions on the benzoates which are practically zero, and in the actions on the isomers ethyl butyrate and isobutyl acetate which are essentially the same. The striking features of these actions may be found in the fact that they are similar in type to the actions of all brain extracts so far studied, including human, beef, and rat, and that they are similar to the embryo type, to the Flexner-Jobling rat carcinoma type, and to a number of tumors of human origin.
The action for the ½ day old rabbit was obtained at the concentration of 8.9 mg. tissue per cc. of solution tested; the remaining actions were obtained at the 17.8 mg. concentration.

With phenyl acetate a small decrease in action was observable as the rabbits became older; with glyceryl triacetate the decrease was very marked. The action on methyl butyrate increased with increase in age; that on ethyl butyrate increased to a small extent. On benzyl acetate, there was a small but distinct decrease. No definite changes could be determined with the remaining esters, possibly because of the small actions.

Heart activity increases as the young animal becomes more active;
at the age of 2 weeks approximately, the animals are able to move about. Before then the motions are more or less uncoordinated. The pictures of the relative actions reflect these changes to a certain extent. It is possible that the decreases in some of the absolute enzyme actions beginning in the early adult life may have some physiological significance.

The absolute actions of the brain extracts are shown in Table VII.

Fig. 8. Spleen Extracts. Five curves are shown on the first plot and four on each of the succeeding. The pictures resemble in general those of the embryo, except that, as with the brain extracts the relative values on the benzoates are very small. There is no real change in type at the different ages.

The first results (−8 days) refer to a concentration of 8.9 mg. tissue per cc. of solution tested, the remaining results to 17.8 mg. per cc.

The interesting feature of these absolute actions is that there is no regular change observable. The actions are practically of the same order of magnitude for each ester throughout. Several series show somewhat greater actions on all the esters, notably those of 28, 67, 1221, and 1737 days of age, but these are isolated and cannot be taken
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as indicative of a regular trend. As stated before, such irregularities are to be expected when dealing with biological material.

The absolute values of the spleen extracts are shown in Table VIII. Because of their small size, it was not possible to study the actions of the spleens in the embryos. The concentrations of the extracts which were tested corresponded to 2.2 mg. tissue per cc. of solution tested. This dilution was much greater than with any other tissue studied. In view of this fact, the enzyme actions found may be said to be quite large. This is of special significance in comparing the brain and spleen actions. The types with these two tissues are very much the same (Figs. 7 and 8), but the absolute actions of the spleens are so much greater that no possibility of confusion exists.

As for the absolute actions of the spleen extracts at the different ages, the actions for the 2½ days and 6½ days rabbits are comparatively

TABLE VIII.

<table>
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<th>Age (days)</th>
<th>PrOAc</th>
<th>G1G0Ac</th>
<th>M1G0C01</th>
<th>PrCHOAc</th>
<th>EtOAc</th>
<th>M1G0Ac</th>
<th>EtOCON</th>
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<td>0.98</td>
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<td>0.64</td>
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<td>0.16</td>
<td>0.50</td>
<td>0.06</td>
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</table>
Fig. 9. Stomach Extracts. These enzyme actions present such an entirely different character that they will be presented in a manner different from the other tissues considered. The absolute actions of these extracts referred to the content of 17.8 mg. tissue per cc. solution are shown. The esters are arranged in the usual order at equidistant intervals along the abscissa axis; the actions corrected for blanks are given as ordinates. The ages of the rabbits are marked on the curves. Four curves are given in the first plot, five in the second.

The striking feature of these results is the increase in actions with the first four esters and ethyl butyrate reaching a maximum at 14 to 28 days and then decreasing again. After the age of 102 days, the actions of the stomach extracts are practically zero for all the esters. While the actions do not increase and decrease in mathematical order as compared to the ages of the rabbits, the general trend is clear and the large actions on certain of the esters, followed by the disappearance of the actions is very definite. The general type of the actions can be seen from the curves although a comparison of the type at the different ages is difficult because of the different magnitudes of the actions. In general, the lack of action on methyl, ethyl and isobutyl acetates, and on the benzoates is significant. In several series of experiments, the enzyme tests were carried out starting at pH 5.0 instead of pH 7.0. The results obtained were similar, so that the actions found do not appear to be due to the hydrogen ion concentration.
small, increasing quite rapidly thereafter. For the first five esters the actions appear to reach a maximum at about the time of sexual maturity, decreasing somewhat thereafter. This trend is clear in spite of certain individual irregularities, especially with the acetates. The actions on the benzoates are practically zero throughout.

In connection with these results it may be of interest to point out that the rabbit spleen is readily autotransplantable in the young rabbit but not in the adult.\(^6\) This is an indication that the spleen is more important to the young rabbit than to the old.

The stomach results raise some interesting physiological questions. In the first place, the study of the ester-hydrolyzing enzymes of the stomach has given at times contradictory results. If the conclusions obtained with rabbits apply to other animals, it is possible that some of the contradictions may be explained as due to the different ages of the animals studied. This age factor has been found to be important with most of the tissues of the rabbit as shown in this paper, but in no case has its significance been as striking as with the stomach. At the same time it must be remembered that there may be a difference in testing the stomach contents and testing the extract of the stomach. The actions found with the nursing rabbits as compared with the adult are of interest in relation to the food consumed, mainly milk with the former, very little or no milk with the latter. If similar enzyme relations were found to hold in man, it would prove of considerable importance in infant nutrition.

**Small Intestine Extracts.**—The intestinal tract was divided into three parts (small intestine, appendix, and large intestine) and their ester-hydrolyzing actions determined in the usual way. The absolute results for the extracts of the appendix and large intestines were very irregular and the pictures of the relative actions while not as irregular as the absolute actions do not show any definite types or changes. They will therefore not be included here and only the results for the small intestine given. The types for the relative actions of the small intestine are in a measure similar to the embryo type but show greater general actions. The results for the butyrates are markedly greater than with the embryo type. The type does not change during the

life cycle of the rabbit as far as these results go. For this reason, the
curves showing the relative actions will be omitted and only the ab-
solute action shown (Table IX).

These actions refer to the concentration of 17.8 mg. per cc. of solu-
tion tested. There appears to be a definite increase in the values
throughout beginning with the 14th day, coincident with the change

<table>
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<th>Age. days</th>
<th>PeOAc</th>
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<th>MoOAc</th>
<th>PhOAc</th>
<th>EIOAc</th>
<th>MOOAc</th>
<th>EEOAc</th>
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<tr>
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from milk to a mixed dietary. The values then remain fairly constant
except for the usual irregularities until with the oldest rabbits the
actions appear to decrease somewhat.

In order to show more clearly the relation between different tissues
at the same ages, the results brought together in Fig. 10 are presented.

The comparative results of the different tissues, as shown in Fig. 10,
may be summarized briefly. The brain does not change in type but
remains embryonic throughout. The liver, between the ages studied, does not show the embryonic type at all. It seems to show the adult type throughout the life cycle except for the reversion in the direction of the embryonic type during the first 4 weeks of life. This reversion,

![Graph showing relative lipase actions of extracts of lung, liver, kidney, brain, and heart muscle of rabbits of different ages. Six different ages are shown, one age to a plot. The ages chosen are 7 days (embryonic), ½ day (after birth), 28 days (dietary fairly mixed, but rabbits not weaned), 195 days and 501 days (adult and sexually mature), 2162 days (old age).]
however, is not enough to overshadow the adult type of action. The kidney clearly shows the embryonic type in the first two plots, gradually changing (28 days), the change being completed in the adult stages. The lung on the other hand, while being close to the embryo type in the first plot (a series obtained at -8 days shown in Fig. 3 but not given in Fig. 10 indicates the embryo type very clearly) changing rapidly and becoming adult in type at or before 28 days. The heart muscle was embryonic in type at \( \frac{1}{2} \) day, the change to adult being only partial at 28 days, but complete at 195 days.

Perhaps the most striking feature of these results is to be found in the fact that different tissues lose their embryonic character as shown by the enzyme actions at different periods of life. In general, those tissues that function least in embryonic life show by their enzyme actions the greatest similarity to the embryo type as well as to certain tumor types. These would include the kidney, skin, and lung. On the other hand the liver, which functions actively in the rabbit embryo behaves chemically as the adult type. Without entering into greater detail, it may be said that there is a striking correlation between the chemical evidence based upon the ester-hydrolyzing enzymes and the functional activity with rabbit tissues.

The question of reversion from the adult type as the rabbit becomes older is of interest. With the whole rat the reversion of the type from the adult picture toward a picture approaching the younger rats, even if not attaining the embryo character was clear and unmistakable. With the rabbit tissues such reversions were not as marked. This may have been due to the ages of the animals. It was possible to obtain rats of extreme old age (i.e., in the neighborhood of three years of age). On the other hand, the oldest rabbits which were available, were five to six years old. If still older rabbits could be secured, say up to eight years of age, the results might be more complete and satisfactory. Even with the rabbits aged five to six years, definite reversions in types and in absolute actions were clearly apparent, although the changes were not as great as with the whole rats. These reversions with the rabbit tissues were given in the consideration of the separate tissues. The results are hardly complete enough to warrant careful comparison and a study of the ages at which the different tissues show such reversion. Except for this brief reference, therefore, these relations will not be considered farther in this connection.
SUMMARY.

The ester-hydrolyzing actions of extracts of a number of tissues of rabbits of different ages were studied under comparable conditions. The ages of the rabbits ranged from 8 days before birth to 2162 days. The esters used included phenyl acetate, glycercyl triacetate, methyl butyrate, benzyl acetate, methyl acetate, ethyl acetate, ethyl butyrate, methyl benzoate, ethyl benzoate, and isobutyl acetate. The following tissues were studied: kidney, liver, lung, skin, leg muscle, heart muscle, brain, spleen, stomach, and small intestine.

The results, as in previous communications, are presented in the form of plots for the relative enzyme actions, and in tables for the absolute actions.

The changes in the curves of the relative actions as the rabbits became older are considered in some detail. The relations between the embryonic state of certain tissues, as shown by their enzyme actions, and the adult state, are described, and compared with their physiological behavior. The probable reversion to a type approaching the embryonic for the oldest rabbits studied is indicated with some of the tissues. The changes in the absolute enzyme actions of the tissues as the rabbits became older are also discussed. The absolute actions do not form as regular a progression as do the relative actions but, at the same time, show marked regularities with increasing age of the rabbits.