TOCOPHEROL CONTENT OF SKELETAL MUSCLE: COMPARISON OF CHEMICAL AND BIOASSAY METHODS*

BY HANS KAUNITZ AND J. J. BEAVER

(From the Departments of Pathology, College of Physicians and Surgeons, and of Chemistry, Columbia University, New York)

(Received for publication, May 29, 1946)

The number of determinations of tocopherols in muscle tissue reported in the literature is small. This may be due to the complexity and time-consuming technique of both the chemical and biological methods, as well as to the poor agreement between the two assays. An accurate account of the chemical assay of vitamin E has recently been compiled in Dr. K. C. D. Hickman's laboratory.¹

The fact that the chemical determinations usually yielded smaller values than the bioassays was probably due to hydrolysis of the extracts to eliminate fats and filtration through an adsorbent earth to separate the tocopherols from carotenes. These procedures were found to be unnecessary if the method of Kaunitz and Beaver (1, 2) for the determination of tocopherols in the presence of fats was applied to muscle extracts.

Extraction of Muscle Tissue

Fig. 1 demonstrates the amounts of fat and tocopherols successively extracted from 97 gm. of human skeletal muscle with 900 ml. of solvent. First, the muscle was emulsified with acetone for 10 minutes in a Waring blender. The volume was made up with acetone in two centrifuge tubes to 1 liter and permitted to stand with occasional shaking; equilibrium was obtained in 24 hours, a time which could have been considerably shortened with a shaking machine. After centrifuging, the fat, tocopherol, and carotene contents of the supernatant fluid were determined by extracting the acetone water layer three times with 300 ml. of purified Skellysolve B, washing the Skellysolve with distilled water, centrifuging, and evaporating under approximately 20 mm. of Hg pressure under N. The residue was weighed, the carotenes were determined by dissolving an aliquot of the residue in a known volume of Skellysolve, and the $-\log T$ value determined in a Coleman universal spectrophotometer or in a Beckman spectrophotometer. The tocopherols were determined according to the method of Kaunitz and Beaver (1, 2) with modifications to be discussed below. The second extraction of the residue of the muscle tissue was a duplicate of

* Aided by a grant from the John and Mary R. Markle Foundation.

¹ Baxter, J. G., Biological symposia, Lancaster, 12, in press.
the first; the others were modified by first using one-half acetone and Skellysolve and then one-third acetone and two-thirds Skellysolve; for the eighth and ninth extractions, concentrated HCl was added as indicated in Fig. 1.

In Fig. 2 are shown the results of successively extracting 6700 gm. of beef muscle with 12 liters of solvent for each extraction. The solvent varied from methyl alcohol to a mixture of one-third methyl alcohol and two-thirds Skellysolve. Concentrated HCl was added for the last two extractions as indicated in Fig. 2.

The data in Figs. 1 and 2 demonstrate that the acetone-Skellysolve mixture removed fat, tocopherol, and carotenes at a more rapid rate than the alcohol-Skellysolve combination, and also that the tocopherol and carotene concentrations roughly parallel the fat concentration curve, except for the first four alcohol concentrations in Fig. 2, in which a relatively higher concentration of tocopherols and carotenes compared to fat was observed. Part of these differences in the rate of extraction is of course due to the change in the solvent-solid ratio.

On the assumption that a distribution coefficient can be calculated between the volume of the emulsified muscle and the volume of the solvent, it is possible to calculate the number of extractions necessary for 98 per cent
extraction of the tocopherols. With a distribution coefficient of 12, the curve indicated in Fig. 1 was obtained. It follows the curve obtained by direct measurements within the experimental error.

On the basis of the above experimental results and calculations, four extractions for at least 24 hours each with a solvent-solid ratio of 20:1, twice with acetone, once with one-half acetone and one-half Skellysolve, and once with one-third acetone and two-thirds Skellysolve, were used as a standard procedure.

![Diagram of extraction process](http://www.jbc.org/)

**Fig. 2.** Total fat, tocopherols, and carotenes obtained by repeated extraction of beef muscle.

**Effect of Carotenes and Vitamin A**

Since carotenes and vitamin A are always present in the tissue extracts and have reducing properties similar to the tocopherols, various procedures have been described to eliminate these substances.

Previous workers have usually sought to accomplish this by filtration through an adsorbent earth (Emmerie and Engel (3); Devlin and Mattill (4); Mayer and Sobotka (5)), advantage being taken of the fact that carotenoids and vitamin A are preferentially adsorbed from benzene solutions. The studies presented here indicate, however, that some tocopherol is
adsorbed on the earth when fat is present. This difficulty has not been sufficiently appreciated.

In previous papers (1, 2), a determination of tocopherols in the presence of fats was described in which the Merck modification (4) of the Emmerie-Engel iron-bipyridine reagent (3) was used. Since fats depress the color formed by this reagent with tocopherol, measurements of the absorption coefficient must be made with and without the addition of known amounts of tocopherol.

This procedure is illustrated in Fig. 3 in which Curve I represents the maximum $-\log T$ values of the bipyridine-fat-tocopherol mixtures necessary to determine $100 \gamma$ of synthetic $dl$-$\alpha$-tocopherol in 100 mg. of sesame

\[ \text{Micrograms } \alpha \text{ tocopherol added} \]

\[ \text{Log } T \]

Fig. 3. Influence of filtration through floridin on tocopherols in the presence of fat. Curves I and II, 1 gm. of sesame oil and 1000 γ of synthetic $dl$-$\alpha$-tocopherol were dissolved in 20 ml. of benzene. One-half of the solution was evaporated, and the residue taken up in 5 ml. of Skellysolve. For Curve II, the benzene mixture was filtered through a 50 × 25 mm. column of HCl-treated floridin, the column washed three times with benzene, the latter evaporated, and the residue treated as for Curve I. 70 γ of $\alpha$-tocopherol out of 100 γ were recovered (for calculation see the equation in "Procedure"). Curves III and IV, a similar experiment with rat fat. From Curves III, the presence of 170 γ of $\alpha$-tocopherol per ml. of Skellysolve can be calculated. Curve IV indicates recovery of 35 γ after floridin filtration.

We are indebted to Hoffmann-La Roche, Inc., for supplying us with $\alpha$-tocopherol.
oil. The method is described below. In Curve II are plotted the results obtained for the same tocopherol-sesame oil mixture after filtration with benzene as solvent through floridin, which had been treated according to Devlin and Mattill (4) with concentrated HCl. From the $-\log T$ values of Curve I, Fig. 3, the presence of 100 $\gamma$ of $\alpha$-tocopherol, as originally added, is calculated from the equation given below (under “Procedure”). Curve II indicated that only 70 $\gamma$ were present after filtration. Despite the loss of 30 per cent of the tocopherol, the $-\log T$ value of the filtered solution, without further tocopherol addition, is greater than that of the original. Since fats, depending on their concentration and physicochemical properties, depress the color (1), the above result indicates that fat was either adsorbed or chemically altered by the floridin.

### Table I

**Influence of Floridin Filtration on Recovery of Tocopherol**

<table>
<thead>
<tr>
<th>Tocopherol used</th>
<th>Amount of fat added</th>
<th>Tocopherol recovered after filtration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>110</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>60</td>
<td>8 (Rat)</td>
<td>80</td>
</tr>
<tr>
<td>60</td>
<td>20 ”</td>
<td>78</td>
</tr>
<tr>
<td>60</td>
<td>40 ”</td>
<td>72</td>
</tr>
<tr>
<td>214</td>
<td>100 ”</td>
<td>52</td>
</tr>
<tr>
<td>60</td>
<td>200 ”</td>
<td>58</td>
</tr>
<tr>
<td>110</td>
<td>400 ”</td>
<td>4</td>
</tr>
<tr>
<td>104</td>
<td>50 (Sesame oil)</td>
<td>62</td>
</tr>
<tr>
<td>428</td>
<td>100 ”</td>
<td>68</td>
</tr>
</tbody>
</table>

Curves III and IV in Fig. 3 present an experiment with rat fat in which 82 per cent of the tocopherol was lost after filtration. The $-\log T$ value after filtration is less than that of the original.

Similar experiments, in which two different floridin samples were used and in which the amounts of fat and of tocopherol were varied, are demonstrated in Table I. The fat and tocopherol were dissolved in purified benzene, and filtered through a floridin column that had been filled with nitrogen. After washing the column with additional benzene, the latter was evaporated in an atmosphere of nitrogen, the residue taken up in purified Skellysolve B, and the tocopherol content of this mixture determined according to the method described below. The recoveries ranged from 4 to 80 per cent, with the greatest losses observed when 100 mg. or more of fat had been added to the benzene.

*Courtesy of the Floridin Company, Warren, Pennsylvania.*
In methods previously used, the muscle extracts were subjected to saponification prior to filtration. The amount of fat present during the filtration was consequently small. One would therefore expect that the losses caused by filtration were not excessive.

These experiments indicate the difficulties encountered when a separation of tocopherols from carotenes is tried in the presence of fats. Since it has been shown previously ((1), p. 654) that tocopherol losses may occur if hydrolysis of the fat is attempted, neither filtration nor hydrolysis seems useful for a quantitative determination of tocopherol in tissue extracts.

When one attempts to determine the reductive capacity of a muscle extract with the iron-bipyridine reagent, accurate results cannot be ob-

![Graph](http://www.jbc.org/)

**Fig. 4.** Curve I, 0.1 ml. of rat muscle extract made to 2 ml. with Skellysolve plus 10 ml. of iron-bipyridine reagent. Curve II, 0.1 ml. of rat muscle extract plus 0.1 ml. of sesame oil made to 2 ml. with Skellysolve plus 10 ml. of iron-bipyridine reagent.

ained because the $-\log T$ value continues to increase for more than 1 hour (Curve I, Fig. 4). If, however, (Curve II) sufficient sesame oil is present in the reacting mixture, a maximum $-\log T$ is reached within 20 minutes. Sesame oil was chosen for these experiments because it usually contains only negligible amounts of reducing substances. Although different samples of the oil produce with the iron-bipyridine reagent different rates of color development for a given amount of tocopherol, or carotenes, the accuracy of the extrapolation method is not affected.

With these facts in mind, correction for the carotenes present in the muscle extract could be made. The determination of the reductive power of carotenes in the absence and in the presence of various fats indicated that
1 γ of carotene is equivalent to 4.8 γ of α-tocopherol; the factors varied from 4.4 to 5.0; since the reductive power of carotenes in a muscle extract does not exceed 10 per cent of the total, the error resulting for the tocopherol from the variations of the carotene factors does not exceed 1 per cent. The total amount of carotenes in the muscle extract was determined by measuring the \(-\log T\) value of the extract at 4400 Å and reading the quantity from a standard curve. Thereafter, 4.8 times the micrograms of carotenes found are deducted from the total reductive power of the extract expressed in micrograms of tocopherol.

A possible error of approximately 2 to 5 per cent is caused by the presence of the normal amounts of vitamin A in the extracts. This estimate is based upon the experimental fact that the reductive power of 1 i. u. of vitamin A was found to be equivalent to 0.24 ± 0.02 γ of α-tocopherol and that 1000 gm. of muscle contain roughly 4000 i. u. of vitamin A. Since it was found that the antimony trichloride method for vitamin A in muscle extracts gave very erratic results, no correction for vitamin A was introduced.

Effect of HCl in Extraction

In experiments reported by Hines and Mattill (6), it was found that, after extraction of rat muscle with alcohol-Skellysolve, further extraction with alcohol-Skellysolve containing HCl brought about an additional yield of 20 to 30 per cent of reducing substance, which they were inclined to believe may have been tocopherol.

As can be seen in Fig. 1, the addition of HCl to the solvent resulted in a further extraction of reducing substance from human muscle, while Fig. 2 shows no further yield from beef muscle under similar conditions.

In these experiments, it was noted that additional reducing substances in the extracts occurred after acid hydrolysis only when a purplish discoloration developed, which was observed especially with rat muscle. Only the colored extracts gave increases up to 25 per cent of the total reducing substances, while the colorless extracts gave none. It therefore seems probable that these additional reducing substances are not tocopherol, and this assumption is supported by the fact that there is good agreement between Mason's (7) bioassays on whole muscles and the values obtained by chemical analysis of muscle extracts without acid hydrolysis (see below).

Method

Reagents

1. Skellysolve B. Purified by twice shaking with concentrated sulfuric acid, washing with water, 10 per cent \(\text{Na}_2\text{CO}_3\), 4 times with water, drying over anhydrous \(\text{Na}_2\text{SO}_4\), and distilling in an all-glass apparatus. A 2 ml. residue from 2 liters gave a \(-\log T\) value of less than 0.002 with the iron-
bipyridine reagent when compared with 2 ml. taken from the solvent as used.


3. Sesame oil. A sample kept in the laboratory for at least several weeks was used. 0.1 ml. of the oil made to 2 ml. with Skellysolve should have a $-\log T$ value of less than 0.002 with 10 ml. of the iron-bipyridine reagent. If the $-\log T$ value is higher, correction has to be made.

4. Iron-bipyridine reagent, as recommended by Merck and Company to Devlin and Mattill (4). 250 mg. of FeCl$_3$·6H$_2$O (reagent grade) and 500 mg. of $\alpha$, $\alpha'$-bipyridine are made to 1 liter with glacial acetic acid.

5. Synthetic dl-$\alpha$-tocopherol.

Procedure—30 to 50 gm. of muscle, weighed to 0.2 gm., are emulsified with about 100 ml. of acetone in a Waring blendor for 6 minutes and washed into a centrifuge tube with an additional 350 ml. of acetone. Determinations can be made with 5 gm. of muscle but with a larger error. After 24 hours, the tube is centrifuged, the residue once more extracted with acetone, once with one-half acetone and one-half Skellysolve, and once with one-third acetone and two-thirds Skellysolve. The combined extracts are transferred to a 3 liter separatory funnel, and water is added until two distinct layers are formed. The Skellysolve phase is saved, the watery phase is twice extracted with 200 ml. of Skellysolve, and the combined Skellysolve extracts are washed three times with 500 ml. of distilled water, centrifuged, and evaporated in an all-glass apparatus under reduced pressure in an atmosphere of nitrogen. The residue is washed into a 25 ml. flask with approximately 20 ml. of Skellysolve; 1 to 2 ml. of sesame oil (according to its color-depressing properties) is added and the volume made to 25 ml. with Skellysolve. The mixture should be slightly yellow and entirely clear. In rare instances, a brown, strongly reducing pigment is noted which interferes with the determination. The pigment can often be removed by washing the Skellysolve extract with a 2 per cent Na$_2$CO$_3$ solution.

For the carotene determination, the $-\log T$ value of the optically clear solution is determined in a Coleman universal spectrophotometer or in a Beckman spectrophotometer with Skellysolve as blank at 4400 Å. The concentration is read from a calibration curve. We are indebted to Dr. Dorothy Andersen and Mrs. Helen Kennedy for a carotene calibration curve.

Two standard solutions containing 40 to 50 and 80 to 100 $\gamma$ of synthetic dl-$\alpha$-tocopherol per ml. of Skellysolve (determined to 1 $\gamma$) are prepared.

Aliquots of the muscle extract of 1 ml. each are pipetted into three test-tubes; to one of these is added 1 ml. of Skellysolve, and to each of the other two 1 ml. of the standard tocopherol solutions. To each test-tube are
added 10 ml. of iron-bipyridine reagent; 2 ml. of Skellysolve containing the same amount of the same sesame oil as the unknown plus 10 ml. of iron-bipyridine reagent serve as a blank. The test-tubes are kept in the dark. Approximately every minute a \(-\log T\) reading is taken, starting with the sample to which the higher tocopherol concentration was added. As soon as the maximum reading is reached, the second tocopherol-containing sample is examined, and finally the sample to which no tocopherol had been added. This procedure is possible because it was found experimentally that the maximum reading appears fastest in the sample with the highest tocopherol concentration. The dilutions of the extracts should be such that none of the \(-\log T\) readings exceeds 0.4.

**Table II**

**Tocopherol Content of Skeletal Muscle (Chemical Analysis)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tocopherol per 1000 gm. wet muscle</th>
<th>Species</th>
<th>Tocopherol per 1000 gm. wet muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From a case of myasthenia gravis.

The uncorrected value, \(x\), for the number of micrograms of tocopherol per ml. of diluted extract is given by the expression \(x = (e_1n)/(e_2 - e_1)\) where \(e_1\) represents the \(-\log T\) value of the unknown, \(e_2\) that of the unknown plus \(n\) \(\gamma\) of tocopherol, and \(n\) the number of micrograms of tocopherol added. For each microgram of carotene per ml. of diluted extract, 4.8 is subtracted from the value for \(x\). All results are finally computed on the basis of mg. of tocopherol per 1000 gm. of muscle.

With different tocopherol additions and different extract concentrations, the maximum errors observed did not exceed \(\pm 10\) per cent.

**Results**

Muscles from humans, rats, and in one case from cattle, have been examined (Table II). For the experiments on humans, the psoas muscle
TOCOPHEROL DETERMINATION IN MUSCLE

was used as soon as possible after the death of the patient. No attention has so far been paid to the cause of death. The rats used had been kept on Rockland pellets throughout life. Any kind of skeletal muscle was used.

The results indicate values considerably higher than those reported by Karrer, Jaeger, and Keller (8), who found 5.9 mg. per 1000 gm. of cattle muscle, and by Hines and Mattill (6), who reported 7.5 mg. of tocopherol per 1000 gm. of rat muscle. In both instances, filtration and saponification of the extracts had been performed and no bioassays were carried out by the authors.

Bioassays—In order to check the above values, bioassays were carried out. Albino rats, whose ancestry had been kept for seven generations on an Evans-Burr diet, were mated, and they and their offspring were placed on the day of birth of a litter on a diet consisting of lard 10 parts, casein (crude) 30 parts, cerelose 54 parts, celluration 2 parts, salt mixture 4 parts, thiamine chloride 2 mg. per kilo, riboflavin 4 mg. per kilo, pyridoxine 4 mg. per kilo, nicotinic acid 100 mg. per kilo, choline 1000 mg. per kilo, vitamin K 4 mg. per kilo, p-aminobenzoic acid 300 mg. per kilo, calcium pantothenate 10 mg. per kilo, and percomorph oil 0.2 ml. per kilo.

The females were used for bioassays after they had reached a weight of 170 gm. or more. They were left for 5 days with the male. Pregnancy occurred in 97 times out of 127 mating experiments in rats of below 6½ months of age. In forty-three tests carried out when these rats were 6½ to 8½ months old, pregnancy was observed thirty-three times. Thereafter, the percentage of pregnancies declined considerably. Only rats of below 6½ months of age were used for the experiments reported in this paper. In the majority of the tests, the desired amount of tocopherol and a comparable dose of muscle extract were fed to a pair of litter mates.

Table III demonstrates the number of litters and of resorptions observed with different tocopherol supplements at different age levels. When the percentage of litters was plotted against the tocopherol supplement, the resulting curves suggested a "mean fertility dose" of about 1.1 mg. of synthetic α-tocopherol for the younger group and about 1.3 mg. for the older ones. In view of the fact that the members of the older group had previously received doses of from 1 to 2 mg. of tocopherol, it was concluded that those previous supplements had no measurable effect on the subsequent tests. The results also indicated that the tocopherol requirements

5 The diet used in these experiments was developed in cooperation with Dr. Charles Slanetz, Department of Animal Care, College of Physicians and Surgeons, Columbia University. We gratefully acknowledge his cooperation in the bioassay work.

6 Per cent composition: NaCl 4.35, MgSO₄ 13.70, NaH₂PO₄ 8.72, K₂HPO₄ 23.98, Ca₃(H₂PO₄)₂ 13.58, ferric citrate 2.97, Ca lactate 32.70.

7 We are indebted to the Abbott Laboratories for the synthetic vitamins.
of the older group were not considerably higher than those of the younger group. It was therefore decided to combine the results of the two groups

| TABLE III |
| Mating Experiments with Tocopherol-Deficient Female Rats at Different Ages |

<table>
<thead>
<tr>
<th>a-Tocopherol supplement (mg.)</th>
<th>Below 4 mos.</th>
<th>4-6½ mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of resorptions</td>
<td>No. of litters</td>
</tr>
<tr>
<td>0.0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1.5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Bioassays with graded doses of tocopherol. ○ represents the results with synthetic dl-a-tocopherol, □ the results with muscle. The figures close to the symbols give the numbers of experiments at the respective levels.

for comparison with the animals that had received muscle extract. The latter were proportionally of the same age as the controls.

On the 5th day after mating had begun, the male was removed and 30
TOCOPHEROL DETERMINATION IN MUSCLE

gm. of the experimental diet containing the tocopherol supplement were offered, which the rats finished in 2 to 3 days. Particular care was taken that the cages were clean when the tocopherol-containing food was offered, and that the rats had completely finished it before the subsequent feeding. The weight of the animals was taken daily, beginning 15 days after mating, and they were examined daily for the presence of a vaginal plug. Only when a vaginal plug was followed by a weight gain and then by weight loss, was it taken as proved that resorption had occurred. The birth of a living litter surviving for at least 1 day was accepted as a positive result.

For the bioassays of the muscle extracts, the tocopherol content of the extracts was calculated according to the chemical test. The extract was diluted with experimental diet, so that 30 gm. contained the desired amount of tocopherol. Otherwise, the animals were treated exactly as the controls that received synthetic dl-a-tocopherol.

No "first litter fertility" was observed (Fig. 5), and at least 2 months were allowed to elapse between tests on the same female; if a litter was born, the rat was not mated for 2 months after the birth of the young.

Fig. 5 demonstrates the results of the bioassays. No litters were observed in eight pregnant rats with 0.5 mg. of synthetic dl-a-tocopherol, or in six that had received muscle extract supposedly containing 0.5 mg. of tocopherol. With 0.75, 1.0, 1.5, and 2.0 mg., the number of litters rose from 38 to 70 per cent. The results suggest a "mean fertility dose" of 1.25 mg. of synthetic dl-a-tocopherol for this particular colony of rats.

With muscle extract, the tocopherol content of which had been determined by the chemical method, the results were found to be within ±10 per cent of those obtained with synthetic dl-a-tocopherol.

DISCUSSION

Mason (7) used whole rat muscle as substrate for tocopherol bioassays. With 60 gm. of muscle of rats kept on dog chow, 50 per cent positive results were observed.

In these experiments, the chemical analysis revealed values of 17 to 30 mg. of tocopherol per 1000 gm. of muscle; the bioassay indicated a "mean fertility dose" of 1.25 mg. of tocopherol, which suggests that 40 to 70 gm. of muscle would yield 50 per cent positive responses. This is in good agreement with Mason's findings, and it is an indication of the fact that complete extraction of the tocopherols is achieved without hydrolysis of the tissue.

The circumstance that the bioassays, for which synthetic dl-a-tocopherol had been used as standard, agree with the chemical analyses indicates that the bulk of the reducing substances in Skellysolve extract of muscle, except for carotenes and vitamin A, is tocopherols and that the bulk of the
tocopherols consists of α-tocopherol. If any considerable amount of the β or γ form were present, no agreement between chemical and bioassay could be expected in view of the much lower biological activity of the latter. 

The agreement between chemical and bioassay methods also suggests that the bulk of the α-tocopherol in the muscle is present in its free, not esterified form, because tocopherol esters would not react with the iron-bipyridine reagent.

SUMMARY

1. A chemical method for the determination of tocopherols in skeletal muscle is described.
2. In human and rat muscle, 17 to 30 mg. of tocopherol per 1000 gm. of wet muscle were recovered.
3. The chemical findings were checked by bioassays; agreement within ±10 per cent between the two methods could be demonstrated.
4. The conclusion seems permissible that the bulk of the reducing substances in a Skellysolve extract of muscle consists, except for carotenes and vitamin A, of non-esterified α-tocopherol.

We wish to thank Dr. Alwin M. Pappenheimer for his suggestions and his unceasing interest in this work, and Miss Ruth Ellen Johnson for technical assistance.

BIBLIOGRAPHY


* Dr. P. L. Harris suggested a different explanation (9). Harris, Jensen, Joffe, and Mason have found that the biological activity of natural α-tocopherols is 50 per cent greater than that of the synthetic α-tocopherol. It is therefore possible that mixtures of natural α-tocopherol with the β and γ forms could give the same effect as synthetic α-tocopherol. While this possibility cannot be ruled out at present, it does not seem probable that mixtures containing the β and γ forms in any considerable amount would give agreement of the chemical and bioassay methods used for these experiments.
TOCOPHEROL CONTENT OF SKELETAL MUSCLE: COMPARISON OF CHEMICAL AND BIOASSAY METHODS
Hans Kaunitz and J. J. Beaver


Access the most updated version of this article at http://www.jbc.org/content/166/1/205.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/166/1/205.citation.full.html#ref-list-1