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# SOME
# STUDIES ON VITAMIN E

BY

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AND

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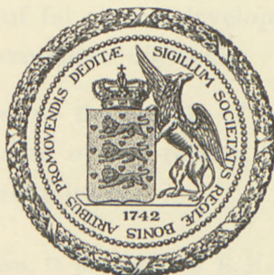


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Previous communications from this laboratory have dealt with two nutritional diseases in chicks, viz. the previously known alimentary encephalomalacia[1] and the new disease alimentary exudative diathesis[2]. It was shown that these diseases are due to a deficiency of vitamin E.

In this paper, our continued studies on vitamin E are reported. The studies can be divided into the following four groups:

1. The chemical determination of tocopherols.
2. Attempts to use the exudative diathesis for the comparison of the activity of natural sources of vitamin E with α-tocopherol.
3. Comparison between the anti-exudative activity of α- and β-tocopherol.
4. The influence of fat on the development of encephalomalacia and exudative diathesis.

This paper presents the results of our studies on these subjects, though some of the results, however, are to be looked upon as preliminary.

[1] H. Dam, J. Glavind, O. Bernth and E. Hagens, Nature **142**, 1157 (1938).

[2] H. Dam and J. Glavind, Nature **142**, 1077, (1938). H. Dam and J. Glavind, Nature **143**, 810 (1939). H. Dam and J. Glavind, Skand. Archiv für Physiol. **82**, 299 (1939). H. Dam and J. Glavind, Die Naturwissenschaften **28**, 207 (1940).

## I. The chemical determination of tocopherols.

For the chemical determination of vitamin E (total amount of tocopherols), several methods are described which we have tried to apply and to improve.

1. The spectrographic method. The use of the absorption spectrum of vitamin E for the chemical determination has been proposed. Cuthbertson et al.[1] used the method for body fat and found that tocopherol could be estimated with reasonable accuracy in crude extract when present in fat in amounts down to 1 %, and in the unsaponifiable fraction when present in amounts down to 0,1 %. A modification of the method consisting in oxidation of the unsaponifiable fraction with silver nitrate made a detection of 0,01 % possible.

We have tried to use the method for pure d,l-α-tocopherol, for wheat germ oil, and for the unsaponifiable fraction of wheat germ oil. The results appear from Fig. 1 which shows the absorption spectra measured by means of a Hilger quartz spectrograph with echelon cells.

Curve 1 shows the absorption spectrum of pure d,l-α-tocopherol in absolute alcohol with a maximum at 293 mμ. Curve 2 gives the absorption spectrum of wheat germ oil in hexane; no maximum at 293 mμ. Curve 3 represents the absorption spectrum of unsaponifiable matter from wheat germ oil in absolute methyl alcohol; it shows a distinct maximum at 293 mμ. The extinction is, however, about 50 % greater than that corresponding to the tocopherol content as determined by the method of Emmerie and Engel. Unsaponifiable matter of extract of alfalfa, even after distillation in a high vacuum, showed no

[1] W. F. J. Cuthbertson, R. R. Ridgeway and J. C. Drummond, Biochem. J. 34, 34 (1940).

maximum at 293 mμ, but a distinct inflection. However, the extinction was only to about 20 % due to tocopherols.

For an exact spectrographic determination of tocopherols, a further purification of the extracts, which is rather difficult, will therefore be necessary. Even if an extinction

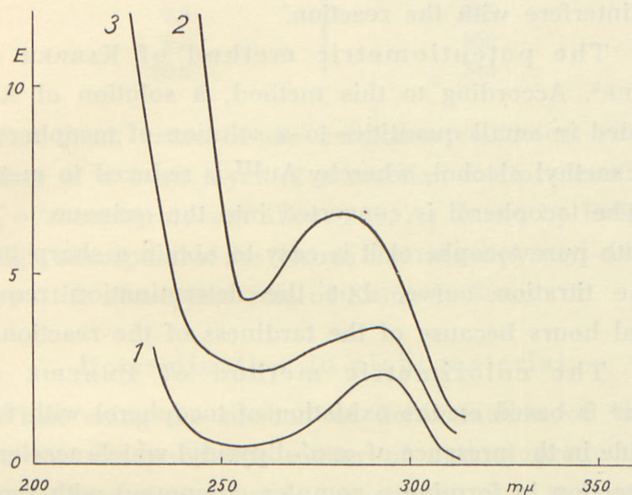


Fig. 1. Abscissae: wave length in m μ.
Ordinates: extinction.
Curve 1: d,l-α-tocopherol 0.032 %.
Curve 2: Wheat germ oil in hexane 10.7 %, corresponding to 0.026 % of tocopherol in the solution.
Curve 3: Unsaponifiable matter from 1.299 g wheat germ oil in 10 cc methyl alcohol corresponding to 0.032 % of tocopherol in the solution.

four times as great is obtained by oxidation with $AgNO_3$, as used by CUTHBERTSON et al., this will not suffice for an exact tocopherol determination in plant material.

2. The colorimetric method of FURTER and MEYER[1]. By means of this method, the red colour developed after heating an alcoholic solution of tocopherol with nitric acid is estimated. We found, however, that this method

[1] M. FURTER and R. E. MEYER, Helv. chim. acta 22, 240 (1939).

was not exact, and the results were more difficult to reproduce than when the method of Emmerie and Engel was applied; therefore we worked exclusively with the last mentioned method. Other authors have found that catechol derivatives[1] and the quinone corresponding to α-tocopherol[2] may interfere with the reaction.

3. The potentiometric method of Karrer and Keller[3]. According to this method, a solution of $AuCl_3$ is added in small quantities to a solution of tocopherol in 80 % methyl alcohol, whereby $Au^{III}$ is reduced to metallic Au. The tocopherol is converted into the quinone.

With pure tocopherol it is easy to obtain a sharp break in the titration curve, but the determination requires several hours because of the tardiness of the reaction.

4. The colorimetric method of Emmerie and Engel[4] is based on the oxidation of tocopherol with ferric chloride in the presence of α:α'-dipyridyl which accelerates the reaction in forming a complex compound with ferrous ions. This complex has a red colour which may be measured after 15 min. by filter S 50 in the Zeiss-Pulfrich photometer. The reaction is carried out in ethyl alcohol which may contain up to 20 % of benzene.

### Determination of the extinction coefficient.

With pure α-tocopherol the method works fairly well. We prepared the α-tocopherol by saponifying the pure acetate and used these solutions for the determination of the extinction coefficient (Table 1).

[1] L. I. Smith, W. B. Irwin and H. F. Ungnade, J. Am. Chem. Soc. 61, 2424 (1939).

[2] A. Emmerie and C. Engel, Rec. trav. chim. 58, 284 (1939).

[3] P. Karrer and H. Keller, Helv. chim. acta 21, 1161 (1938).

[4] A. Emmerie and C. Engel, Rec. trav. chim. 57. 1351 (1938).

Table 1

| mg d,l-α-tocopherol acetate saponified | Extinction coefficient $E^{1\,cm}_{1\,\%}$ |
|---|---|
| 268.3 | 345 |
| 9.8 | 335 |
| 9.8 | 350 |
| 10.6 | 344 |

The mean value of the extinction coefficient (the extinction of a 1 cm layer of a reaction mixture containing 1 % of tocopherol) was about 345. As shown by EMMERIE, the light absorption of the ferrous chloride-dipyridyl-complex follows the absorption rule of Lambert-Beer.

## Determination in plant material.

When using the method of determination of vitamin E in the unsaponifiable matter of natural products, the same sources of error are to be expected as in the potentiometric method.

EMMERIE and ENGEL[1] proposed to remove carotenoids by adsorption on columns of floridin XS earth. The carotenoids are said to be adsorbed while tocopherol is passing into the filtrate. We were not able to obtain reliable results in this way.

A more suitable method to eliminate carotenoids and certain other reducing substances is the acetylation method developed by KARRER and KELLER[2] in their potentiometric method. They heated a mixture of β-carotene and tocopherol with acetic anhydride in pyridine solution on the water bath for two hours. The pyridine solution is then

[1] A. EMMERIE and C. ENGEL, Rec. trav. chim. 58, 3 (1939).

[2] P. KARRER and H. KELLER, Helv. chim. acta 22, 253 (1939).

diluted with petrol ether, washed with diluted hydrochloric acid, and then with water until neutral reaction. Then the petrol ether is evaporated and the remaining substances are titrated with auric chloride. Another sample of the carotene-tocopherol-mixture is titrated without acetylation and the tocopherol content is evaluated as the difference between the two titrations. Karrer and Keller found that by this method a fairly good separation between tocopherol and carotene is obtained. We carried out similar experiments with mixtures of carotene and tocopherol and carotene alone, but we made the determinations by means of the colorimetric method. We were able to state the results of Karrer and Keller and, consequently, to include an acetylation in our tocopherol determination. We found it to be important to use freshly distilled pyridine and to carry out the acetylation in a $N_2$-atmosphere.

We obtained further separation from other reducing non-tocopherols by including in the determination a distillation in a high vacuum. According to Emerson et al.[1], the tocopherols distill at $10^{-5}$ mm Hg between 110° and 140°. Our experiments revealed the fact that the unsaponifiable matter of certain natural products, for instance the lipoids of green leaves and hips, contained appreciable amounts of substances which reduce ferric chloride, but do not distill in high vacuum as vitamin E does.

We distilled pure α-tocopherol and mixtures of α-tocopherol and β-carotene for 5 minutes in high vacuum at 140° in the apparatus described below (Fig. 2) and determined the tocopherol-content of the distillate colorimetrically (Table 2).

[1] O. H. Emerson, G. A. Emerson, A. Mohammed and H. M. Evans, J. Biol. Ch. **122**, 99 (1937).

Table 2.

| Product distilled | Per cent recovery |
|---|---|
| 1.9 mg d, l-α-tocopherol ...................... | 96 |
| 1.9 - d, l-α-tocopherol ...................... | 97 |
| 1.9 - d, l-α-tocopherol + 0,17 mg β-carotene.. | 95 |
| 0.95 - d, l-α-tocopherol + 0,68 - β-carotene.. | 98 |

Distillation longer than 5 minutes did not yield greater recovery. By distillation of unsaponifiable matter from wheat germ oil the following results were obtained (Table 3). The distillation lasted for 10 minutes.

Table 3.

| Product distilled | Per cent recovery |
|---|---|
| 8 mg of unsaponifiable matter of wheat germ oil | 94 |
| 45 - - — — - — — - | 91 |
| 7 - - — — - — — - | 98 |
| 54 - - — — - — — - | 94 |

As "per cent recovery" we denote tocopherol colorimetrically determined in the distillate in relation to tocopherol colorimetrically determined before the distillation. Also here, almost total recovery was obtained if amounts not greater than 10—20 mg were distilled. After acetylation of the unsaponifiable matter of wheat germ oil, practically no reduction power remains.

The unsaponifiable matter of the lipoids of hips and green leaves behaves in quite a different way. Here, a correction of 10—50 % can be obtained by the acetylation method, and a considerably greater correction will be obtained by subtracting that part of the reducing substances which remains in the non-distillable fraction (Table 4).

Table 4.

| Product distilled | Colorimetrically determined value calculated as $^0/_0$ tocopherol in dried material | | |
|---|---|---|---|
| | direct determ-ination | after subtract-ion for acetylation | in the distillate |
| 4.0 mg of unsaponifi-able of hips | 0.056 | 0.048 | 0.032 |
| 4.9 mg — — | 0.044 | 0.033 | 0.022 |
| 6.0 mg — — | 0.045 | 0.034 | 0.025 |
| 5.0 mg of unsaponifi-able of spinach | 0.261 | 0.142 | 0.045 |
| 5.2 mg — — | 0.272 | 0.170 | 0.062 |
| 3.0 mg of unsaponifi-able of alfalfa | 0.192 | 0.138 | 0.044 |
| 2.0 mg — — | 0.144 | 0.960 | 0.040 |

These results suggest the assumption that certain components hitherto unknown must be present in unsaponifiable matter from lipoids of hips and dried leaves, since, if a known amount of d, l-α-tocopherol is added before the distillation, the total amount of tocopherol can be recovered on the condenser and, furthermore, the total reduction power of distillate + non-distillable is the same as before the distillation.

It might be of interest to investigate the chemical nature of these unknown reducing substances. They will probably be alcohols, since their reducing power is eliminated by acetylation. They have no vitamin E activity when tested on chicks (these studies are reported in Part II of this paper).

If the method is to be applied on animal material, it must be reminded that vitamin A which does not occur in plant material will also reduce ferric chloride. Vita-

min A distills in high vacuum, and the reduction power is diminished by acetylation, however, it may possess part of its reducing properties even after acetylation. Vitamin A is, therefore, not eliminated by the method.

Perhaps, it becomes possible to eliminate vitamin A by means of the antimony trichloride reaction, such as proposed by KARRER and KELLER[1].

Frequently, but a very small acetylation correction is obtained in the distillate; sometimes, this correction may amount to 10 %. It is, therefore, advisable in every case to examine whether an acetylation after the distillation is necessary or not.

## Procedure for tocopherol-determination in dried plant material.

Production of the unsaponifiable matter. About 5 grams of the material are weighed and ground in a mortar with about equal parts of pulverized quartz and a little $CaCO_3$. The mixture is extracted on a glass filter with 33 % freshly distilled acetone; this extract is removed. The main extraction is carried out by means of successive portions of pure acetone, until the acetone remains colourless. Then one extraction with 90 % acetone and two extractions with ether are carried out. The combined extracts are evaporated in vacuo at about 40°. The residue is transferred to a separatory funnel by means of ether and separated from eventually admixed water. Then the ether is dried with $Na_2SO_4$ overnight; the $Na_2SO_4$ is filtered off, washed with ether and the combined extracts are evaporated in vacuo to about 25 cc. The ether solution is then saponified with 4 n methyl alcoholic potassium hydroxide in an $N_2$ atmosphere in a

[1] loc. cit. p. 7.

separatory funnel for two hours. The mixture is diluted with water, the watery phase is separated and washed with ether. The combined ether phases are washed with water until they are alkali free and are dried over $Na_2SO_4$. After filtration and evaporation in vacuo, the residue is soluted in ethyl alcohol plus a little benzene.

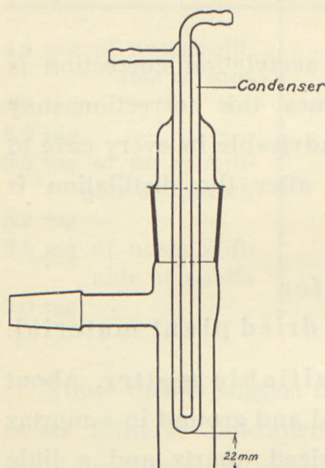

Fig. 2.

Distillation. As much of this solution as contains 5—10 mg of dried material is carefully evaporated on the bottom of the vacuum still (Fig. 2) which is thereafter connected to the high vacuum pump aggregate consisting of a mercury diffusion pump, a condenser immersed in acetone-solid $CO_2$ and a mechanical fore-vacuum pump. When the pressure has fallen below $10^{-3}$ mm Hg, measured by means of a v. Reden manometer, the apparatus is immersed into liquid Wood's metal at 140° so that the surface of the metal is 2.5 cm above the deepest point of the condenser. This temperature is maintained for 25—30 minutes. Then, the metal bath is removed and the apparatus is allowed to cool. The distillate is collected from the condenser by washing with ethanol-benzene.

Acetylation. One part of the distillate is used directly for the colorimetric reaction. The rest is evaporated, soluted in 1 cc acetic anhydride + 5 cc freshly distilled pyridine and the acetylation is carried on for two hours in an $N_2$-atmosphere on a boiling water bath with reflux-condenser. The solution is then diluted with petrol ether, and the

petrol ether layer is washed, first with diluted hydrochloric acid and then with water until neutral. The petrol ether is dried with $Na_2SO_4$, evaporated and soluted in ethanol-benzene.

The colour reaction. As much of the ethanol-benzene solution as contains about 0.1 mg of tocopherol + 1 cc 0.5 % $\alpha, \alpha'$-dipyridyl in ethanol + 1 cc 0.2 % $FeCl_3$ in ethanol is transferred to a 25 cc measuring flask and diluted to the mark with ethanol. The photometer is read after 15 minutes by screen S 50 and 5 cm microcuvettes. The result is calculated by means of the extinction coefficient for pure d, l-$\alpha$-tocopherol which is 345, as mentioned above.

The tocopherol content of some natural products.

By means of this method, the tocopherol content of a series of natural products was determined (Table 5). It is interesting to see that dried green plants such as spinach, alfalfa, and dried hips contain just as much tocopherol as wheat germ, hitherto looked upon as the most vitamin E-potent source. Lipoid extracts of these products contain much more tocopherol than wheat germ oil. The high

Table 5.

| Product | % tocopherol |
|---|---|
| Wheat germ (after the season) ................. | 0.015—0.038 |
| Wheat germ oil (after the season), pressed .... | 0.14— 0.25 |
| Wheat germ oil, extracted with petrol ether... | 0.45 |
| Wheat germ oil of the same charge pressed ... | 0.23 |
| Dried hips (without pips) ..................... | 0.02— 0.05 |
| Petrol ether extract of dried hips........about | 2.3 |
| Dried spinach ............................ — | 0.05 |
| Dried alfalfa (commercial products) ........... | 0.01— 0.045 |
| Apple pips .............................. about | 0.02 |
| Mangold ............................. less than | 0.00001 |

potency of these products as vitamin E sources has been confirmed in animal experiments (Part II).

### Summary.

Various chemical measures for the determination of vitamin E (tocopherols) have been examined. The best results were obtained by means of the α, α′-dipyridyl-ferric chloride method after the product had been purified by saponification and distillation in a high vacuum.

## II. Some experiments on estimating the vitamin E activity of natural products by means of alimentary exudative diathesis.

In our previous works on vitamin E, we suggested to use the antiencephalomalacia activity or the antiexudation activity of vitamin E for its biological estimation. Such experiments have now been carried out.

Experimental. Chicks weighing about 100 g were kept as usual in individual cages under clean conditions. They were fed the vitamin E-free diet 545 consisting of diet 538 + 5 $^0/_0$ cod liver oil.

Diet 538

| | |
|---|---|
| Casein, alcohol extracted | 150 |
| Dried brewer's yeast | 100 |
| Sugar | 730 |
| Salt mixture No. 3*) | 20 |
| | 1000 |

*) Salt mixture No. 3

| | |
|---|---|
| Calcium carbonate | 400 |
| Magnesium carbonate | 20 |
| Sodium chloride | 88 |
| Ferric citrate | 32 |
| Di-jodo-tyrosine | 0.005 |
| Cupric sulphate, cryst. | 3 |
| Manganous sulphate, cryst. | 6 |
| | 549 |

Vitamin K was furnished in the form of tablets containing a highly purified product, resp. pure $K_1$, six times

a week. The substances, whose vitamin E activity had to be tested, resp. pure synthetic d,l-α-tocopherol acetate (kindly put at our disposal by F. Hoffmann-la Roche & Co. A.G., Basel), which was used as standard, were also furnished as tablets six times a week.

Three series of experiments, A, B and C, were carried out.

In series A, the effects of a logarithmic series of doses of dried hips were compared with a logarithmic series of doses of tocopherol. The following doses were used: 0.25 — 0.5 — 1 and 2 g of dried hips and 0.1 — 0.2 — 0.4 — 0.8 — 1.6 and 3.2 mg of d, l-α-tocopherol acetate.

In series B, the effects of varying doses of dried hips were tried again, but smaller doses were used, viz. 60 — 120 and 240 mg. At the same time, the effect of an acetone extract of the dried hips, from which tablets were made by means of lactose and talc, was tested. These tablets were fed in such amounts that the doses corresponded to 60 — 120 and 240 mg of dried hips. From d, l-α-tocopherol acetate doses of 0.1 — 0.2 and 0.4 mg were used.

The experiments in series C were carried out in order to settle the question whether the reducing but non-distilling substances from alfalfa, described in Part I, had any E activity. One group of animals received every day (except Sunday) 1 tablet containing as much of the distillate at 140° and $10^{-3}$ mm Hg of unsaponifiable matter of alfalfa as, on the basis of the chemical determination, corresponded to 0.4 mg of d, l-α-tocopherol acetate; the second group was fed the corresponding amount of non-distilling product. Further groups got no tablets and 0.1 — 0.2 — 0.4 mg of d, l-α-tocopherol acetate.

The animals were carefully inspected for exudates and encephalomalacia every day. Encephalomalacia was dia-

gnozed by the behaviour of the animals and by the macroscopic findings on opening the brain. After 35—40 days, the surviving animals were killed. Autopsy was made in every case. The results appear from Table 1. In this table, exudations as well as encephalomalacias are indicated by + in the respective columns. As mentioned in our previous papers, the hemorrhagic condition of the fat tissue seen by autopsy is part of the exudative symptom. Where hemorrhage in fat tissue occurred without exudate, the case was considered to be the initial stage of the full symptom. In Table 1, such cases are indicated by (+); by the statistical evaluation (Table 2) they are arbitrarily calculated as $^1/_2$ full exudation. Table 1 contains, furthermore, the time until the appearence of the exudates as well as the number of days from the beginning of the experiment until the chick died or was killed. Animals which were killed are marked with k, and those which died during the feeding period are marked with d.

The results are summarized in Table 2 which indicates the percentage of animals showing exudates resp. encephalomalacia. Animals which died before the 7th day of the experiment are left out in the calculation.

Table 1.
Series A. Started 28-29/9 1939

| Group 1 no addition | | | | | | Group 2 0.1 mg d,l-α-tocopherol acetate p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 5931 | 27 d | + | 22 | + | 14 | 5821 | 25 k | (+) | .. | 0 | .. |
| 5932 | 24 d | + | 20 | 0 | .. | 5822 | 36 k | 0 | .. | 0 | .. |
| 5933 | 4 d | 0 | .. | 0 | .. | 5823 | 35 k | 0 | .. | 0 | .. |
| 5934 | 35 k | + | 16 | 0 | .. | 5824 | 13 k | + | 11 | 0 | .. |
| 5935 | 22 d | + | 8 | 0 | .. | 5825 | 35 k | 0 | .. | 0 | .. |
| 5936 | 12 d | + | 12 | 0 | .. | 5826 | 35 k | 0 | .. | 0 | .. |
| 5937 | 7 d | 0 | .. | 0 | .. | 5827 | 35 k | + | 11 | 0 | .. |
| 5938 | 14 d | + | 12 | 0 | .. | 5828 | 36 k | (+) | .. | 0 | .. |
| 5939 | 8 d | + | 8 | + | 8 | 5829 | 33 d | (+) | .. | 0 | .. |
| 5940 | 8 k | + | 8 | + | 8 | 5830 | 36 k | + | 11 | 0 | .. |
| Group 3 0.2 mg d,l-α-tocopherol acetate p. day | | | | | | Group 4 0.4 mg d,l-α-tocopherol acetate p. day | | | | | |
| 5831 | 37 k | + | 26 | 0 | .. | 5841 | 35 k | 0 | .. | 0 | .. |
| 5832 | 35 k | 0 | .. | 0 | .. | 5842 | 35 k | 0 | .. | 0 | .. |
| 5833 | 22 k | (+) | .. | 0 | .. | 5843 | 35 k | 0 | .. | 0 | .. |
| 5834 | 35 k | 0 | .. | 0 | .. | 5844 | 36 k | 0 | .. | 0 | .. |
| 5835 | 35 k | 0 | .. | 0 | .. | 5845 | 35 k | 0 | .. | 0 | .. |
| 5836 | 36 k | 0 | .. | 0 | .. | 5846 | 36 k | 0 | .. | 0 | .. |
| 5837 | 35 k | 0 | .. | 0 | .. | 5847 | 36 k | 0 | .. | 0 | .. |
| 5838 | 37 k | + | 20 | 0 | .. | 5848 | 36 k | 0 | .. | 0 | .. |
| 5839 | 36 k | 0 | .. | 0 | .. | 5849 | 37 k | 0 | .. | 0 | .. |
| 5840 | 36 k | + | 19 | 0 | .. | 5850 | 37 k | 0 | .. | 0 | .. |
| Group 5 0.8 mg d,l-α-tocopherol acetate p. day | | | | | | Group 6 1.6 mg d,l-α-tocopherol acetate p. day | | | | | |
| 5851 | 35 k | 0 | .. | 0 | .. | 5861 | 35 k | 0 | .. | 0 | .. |
| 5852 | 35 k | 0 | .. | 0 | .. | 5862 | 35 k | 0 | .. | 0 | .. |
| 5853 | 36 k | 0 | .. | 0 | .. | 5863 | 35 k | 0 | .. | 0 | .. |
| 5854 | 35 k | 0 | .. | 0 | .. | 5864 | 36 k | 0 | .. | 0 | .. |
| 5855 | 35 k | 0 | .. | 0 | .. | 5865 | 35 k | 0 | .. | 0 | .. |
| 5856 | 12 d | 0 | .. | 0 | .. | 5866 | 36 k | 0 | .. | 0 | .. |
| 5857 | 36 k | 0 | .. | 0 | .. | 5867 | 36 k | 0 | .. | 0 | .. |
| 5858 | 36 k | 0 | .. | 0 | .. | 5868 | 36 k | 0 | .. | 0 | .. |
| 5859 | 36 k | 0 | .. | 0 | .. | 5869 | 35 d | 0 | .. | 0 | .. |
| 5860 | 37 k | 0 | .. | 0 | .. | 5870 | 6 d | 0 | .. | 0 | .. |

Table 1 (continued).

| Group 7 3.2 mg d,l-α-tocopherol acetate p. day | | | | | | Group 8 0.25 g dried hips p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 5871 | 35 k | 0 | .. | 0 | .. | 5881 | 35 k | 0 | .. | 0 | .. |
| 5872 | 35 k | 0 | .. | 0 | .. | 5882 | 35 k | (+) | .. | 0 | .. |
| 5873 | 35 k | 0 | .. | 0 | .. | 5883 | 35 k | 0 | .. | 0 | .. |
| 5874 | 36 k | 0 | .. | 0 | .. | 5884 | 35 k | 0 | .. | 0 | .. |
| 5875 | 27 d | 0 | .. | 0 | .. | 5885 | 15 d | (+) | .. | 0 | .. |
| 5876 | 36 k | 0 | .. | 0 | .. | 5886 | 36 k | 0 | .. | 0 | .. |
| 5877 | 36 k | 0 | .. | 0 | .. | 5887 | 36 k | 0 | .. | 0 | .. |
| 5878 | 36 k | 0 | .. | 0 | .. | 5888 | 37 k | (+) | .. | 0 | .. |
| 5879 | 37 k | 0 | .. | 0 | .. | 5889 | 37 k | 0 | .. | 0 | .. |
| 5880 | 36 k | 0 | .. | 0 | .. | 5890 | 37 k | 0 | .. | 0 | .. |
| Group 9 0.5 g dried hips p. day | | | | | | Group 10 1 g dried hips p. day | | | | | |
| 5891 | 35 k | 0 | .. | 0 | .. | 5901 | 35 k | 0 | .. | 0 | .. |
| 5892 | 35 k | 0 | .. | 0 | .. | 5902 | 36 k | 0 | .. | 0 | .. |
| 5893 | 35 k | 0 | .. | 0 | .. | 5903 | 35 k | 0 | .. | 0 | .. |
| 5894 | 35 k | (+) | .. | 0 | .. | 5904 | 35 k | 0 | .. | 0 | .. |
| 5895 | 36 k | 0 | .. | 0 | .. | 5905 | 36 k | 0 | .. | 0 | .. |
| 5896 | 36 k | 0 | .. | 0 | .. | 5906 | 35 k | 0 | .. | 0 | .. |
| 5897 | 37 k | 0 | .. | 0 | .. | 5907 | 15 d | (+) | .. | 0 | .. |
| 5898 | 37 k | 0 | .. | 0 | .. | 5908 | 2 d | 0 | .. | 0 | .. |
| 5899 | 37 k | 0 | .. | 0 | .. | 5909 | 36 k | 0 | .. | 0 | .. |
| 5900 | 11 d | 0 | .. | 0 | .. | 5910 | 36 k | 0 | .. | 0 | .. |
| Group 11 2 g dried hips p. day | | | | | | | | | | | |
| 5912 | 35 k | 0 | .. | 0 | .. | | | | | | |
| 5913 | 35 k | 0 | .. | 0 | .. | | | | | | |
| 5914 | 35 k | 0 | .. | 0 | .. | | | | | | |
| 5915 | 3 d | 0 | .. | 0 | .. | | | | | | |
| 5916 | 35 k | 0 | .. | 0 | .. | | | | | | |
| 5917 | 36 k | 0 | .. | 0 | .. | | | | | | |
| 5919 | 20 d | 0 | .. | 0 | .. | | | | | | |
| 5920 | 36 k | 0 | .. | 0 | .. | | | | | | |
| 5922 | 37 k | 0 | .. | 0 | .. | | | | | | |
| 5925 | 37 k | 0 | .. | 0 | .. | | | | | | |
| 5926 | 37 k | 0 | .. | 0 | .. | | | | | | |
| 5927 | 37 k | 0 | .. | 0 | .. | | | | | | |

Table 1 (continued).

Series B. Started 20/11-1939.

| Group 1 no addition | | | | | | Group 2 0.1 mg d,l-α-tocopherol acetate p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 5961 | 28 k | + | 28 | + | 16 | 5971 | 40 k | 0 | .. | 0 | .. |
| 5962 | 24 k | + | 16 | 0 | .. | 5972 | 40 k | + | 40 | 0 | .. |
| 5963 | 14 d | + | 14 | 0 | .. | 5973 | 40 k | 0 | .. | 0 | .. |
| 5964 | 16 k | 0 | .. | 0 | .. | 5974 | 40 k | + | 29 | + | 35 |
| 5965 | 40 k | + | 40 | 0 | .. | 5975 | 43 k | 0 | .. | 0 | .. |
| 5966 | 16 d | + | 14 | 0 | .. | 5976 | 43 k | 0 | .. | 0 | .. |
| 5967 | 25 k | + | 14 | + | 19 | 5977 | 43 k | 0 | .. | 0 | .. |
| 5968 | 12 d | (+) | .. | 0 | .. | 5978 | 43 k | 0 | .. | 0 | .. |
| 5969 | 12 d | (+) | .. | 0 | .. | 5979 | 43 k | + | 25 | 0 | .. |
| 5970 | 40 k | 0 | .. | 0 | .. | 5980 | 43 k | + | 10 | 0 | .. |
| Group 3 0.2 mg d,l-α-tocopherol acetate p. day | | | | | | Group 4 0.4 mg d,l-α-tocopherol acetate p. day | | | | | |
| 5981 | 40 k | 0 | .. | 0 | .. | 5991 | 40 k | 0 | .. | 0 | .. |
| 5982 | 40 k | + | 29 | 0 | .. | 5992 | 40 k | 0 | .. | 0 | .. |
| 5983 | 40 k | 0 | .. | 0 | .. | 5993 | 40 k | 0 | .. | 0 | .. |
| 5984 | 43 k | 0 | .. | 0 | .. | 5994 | 38 k | 0 | .. | 0 | .. |
| 5985 | 43 k | (+) | .. | 0 | .. | 5995 | 38 k | 0 | .. | 0 | .. |
| 5986 | 43 k | 0 | .. | 0 | .. | 5996 | 40 k | 0 | .. | 0 | .. |
| 5987 | 43 k | (+) | .. | 0 | .. | 5997 | 38 k | 0 | .. | 0 | .. |
| 5988 | 38 k | + | 38 | 0 | .. | 5998 | 38 k | 0 | .. | 0 | .. |
| 5989 | 43 k | 0 | .. | 0 | .. | 5999 | 43 k | 0 | .. | 0 | .. |
| 5990 | 43 k | 0 | .. | 0 | .. | 7000 | 42 k | 0 | .. | 0 | .. |
| Group 5 1 tablet of dried hips (60 mg) p. day | | | | | | Group 6 2 tablets of dried hips (60 mg each) p. day | | | | | |
| 7001 | 40 k | 0 | .. | 0 | .. | 7011 | 40 k | + | 30 | 0 | .. |
| 7002 | 40 k | 0 | .. | + | 25 | 7012 | 10 d | + | 8 | 0 | .. |
| 7003 | 40 k | 0 | .. | 0 | .. | 7013 | 2 d | 0 | .. | 0 | .. |
| 7004 | 38 k | + | 19 | 0 | .. | 7014 | 40 k | 0 | .. | 0 | .. |
| 7005 | 20 d | 0 | .. | + | 19 | 7015 | 40 k | 0 | .. | 0 | .. |
| 7006 | 42 k | 0 | .. | 0 | .. | 7016 | 41 d | + | 39 | 0 | .. |
| 7007 | 38 k | + | 31 | 0 | .. | 7017 | 42 k | (+) | .. | 0 | .. |
| 7008 | 42 k | 0 | .. | 0 | .. | 7018 | 10 k | + | 9 | 0 | .. |
| 7009 | 42 k | 0 | .. | 0 | .. | 7019 | 42 k | 0 | .. | 0 | .. |
| 7010 | 42 k | + | 22 | 0 | .. | 7020 | 42 k | 0 | .. | 0 | .. |

Table 1 (continued).

| Group 7 4 tablets of dried hips (60 mg each) p. day | | | | | | Group 8 1 tablet (60 mg) of extract of dried hips p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 7021 | 40 k | (+) | .. | 0 | .. | 7031 | 22 d | 0 | .. | + | 10 |
| 7022 | 24 k | + | 22 | 0 | .. | 7032 | 13 d | + | 13 | 0 | .. |
| 7023 | 40 k | 0 | .. | 0 | .. | 7033 | 40 k | 0 | .. | 0 | .. |
| 7024 | 40 k | + | 30 | 0 | .. | 7034 | 40 k | 0 | .. | 0 | .. |
| 7025 | 42 k | 0 | .. | 0 | .. | 7035 | 37 k | + | 36 | 0 | .. |
| 7026 | 42 k | + | 42 | 0 | .. | 7036 | 40 k | 0 | .. | + | 23 |
| 7027 | 42 k | 0 | .. | 0 | .. | 7037 | 42 k | 0 | .. | 0 | .. |
| 7028 | 28 k | + | 23 | 0 | .. | 7038 | 42 k | + | 22 | 0 | .. |
| 7029 | 42 k | + | 39 | 0 | .. | 7039 | 16 k | + | 9 | + | 10 |
| 7030 | 42 k | 0 | .. | 0 | .. | 7040 | 42 k | + | 24 | 0 | .. |

| Group 9 2 tablets (60 mg each) of extract of dried hips p. day | | | | | | Group 10 4 tablets (60 mg each) of extract of dried hips p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7041 | 10 k | + | 8 | 0 | .. | 7051 | 40 k | 0 | .. | 0 | .. |
| 7042 | 40 k | 0 | .. | 0 | .. | 7052 | 7 d | 0 | .. | 0 | .. |
| 7043 | 40 k | 0 | .. | 0 | .. | 7053 | 38 k | + | 38 | 0 | .. |
| 7044 | 40 k | 0 | .. | 0 | .. | 7054 | 40 k | 0 | .. | 0 | .. |
| 7045 | 42 k | + | 37 | 0 | .. | 7055 | 40 k | (+) | .. | 0 | .. |
| 7046 | 26 k | + | 15 | 0 | .. | 7056 | 42 k | (+) | .. | 0 | .. |
| 7047 | 42 k | 0 | .. | 0 | .. | 7057 | 42 k | 0 | .. | 0 | .. |
| 7048 | 42 k | 0 | .. | 0 | .. | 7058 | 21 d | 0 | .. | + | 19 |
| 7049 | 42 k | 0 | .. | + | 18 | 7059 | 42 k | (+) | .. | 0 | .. |
| 7050 | 42 k | + | 23 | 0 | .. | 7060 | 42 k | 0 | .. | 0 | .. |

Series C. Started 16/5-1940.

| Group 1 no addition | | | | | | Group 2 0.1 mg d,l-α-tocopherol acetate p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9080 | 39 d | + | 31 | 0 | .. | 9090 | 39 k | 0 | .. | 0 | .. |
| 9081 | 34 d | + | 27 | 0 | .. | 9091 | 39 k | 0 | .. | 0 | .. |
| 9082 | 39 k | 0 | .. | 0 | .. | 9092 | 39 k | 0 | .. | 0 | .. |
| 9083 | 39 k | 0 | .. | 0 | .. | 9093 | 35 d | 0 | .. | 0 | .. |
| 9084 | 39 k | + | 39 | 0 | .. | 9094 | 39 k | 0 | .. | 0 | .. |
| 9085 | 39 k | + | 27 | + | 36 | 9095 | 39 k | 0 | .. | 0 | .. |
| 9086 | 36 k | + | 29 | 0 | .. | 9096 | 40 k | + | 29 | 0 | .. |
| 9087 | 30 d | + | 16 | 0 | .. | 9097 | 40 k | 0 | .. | 0 | .. |
| 9088 | 40 k | 0 | .. | 0 | .. | 9098 | 40 k | 0 | .. | 0 | .. |
| 9089 | 40 k | 0 | .. | 0 | .. | 9099 | 40 k | 0 | .. | 0 | .. |

Table 1 (continued).

| Group 3 0.2 mg d,l-α-tocopherol acetate p. day | | | | | | Group 4 0.4 mg d,l-α-tocopherol acetate p. day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 9100 | 39 k | 0 | .. | 0 | .. | 9110 | 39 k | 0 | .. | 0 | .. |
| 9101 | 39 k | 0 | .. | 0 | .. | 9111 | 39 k | 0 | .. | 0 | .. |
| 9102 | 39 k | 0 | .. | 0 | .. | 9112 | 39 k | 0 | .. | 0 | .. |
| 9103 | 39 k | 0 | .. | 0 | .. | 9113 | 29 d | 0 | .. | 0 | .. |
| 9104 | 39 k | 0 | .. | 0 | .. | 9114 | 39 k | 0 | .. | 0 | .. |
| 9105 | 39 k | 0 | .. | 0 | .. | 9115 | 39 k | 0 | .. | 0 | .. |
| 9106 | 40 k | 0 | .. | 0 | .. | 9116 | 40 k | 0 | .. | 0 | .. |
| 9107 | 40 k | 0 | .. | 0 | .. | 9117 | 40 k | 0 | .. | 0 | .. |
| 9108 | 40 k | 0 | .. | 0 | .. | 9118 | 40 k | 0 | .. | 0 | .. |
| 9109 | 40 k | 0 | .. | 0 | .. | 9119 | 40 k | 0 | .. | 0 | .. |
| Group 5 distillate from alfalfa | | | | | | Group 6 undistillable from alfalfa | | | | | |
| 7441 | 39 k | 0 | .. | 0 | .. | 7453 | 39 k | + | 27 | 0 | .. |
| 7442 | 39 k | 0 | .. | 0 | .. | 7454 | 39 k | + | 32 | 0 | .. |
| 7443 | 38 d | 0 | .. | 0 | .. | 7455 | 39 k | + | 25 | 0 | .. |
| 7444 | 39 k | 0 | .. | 0 | .. | 7456 | 39 k | + | 28 | 0 | .. |
| 7445 | 30 d | 0 | .. | 0 | .. | 7457 | 39 k | 0 | .. | 0 | .. |
| 7446 | 39 k | 0 | .. | 0 | .. | 7458 | 39 k | 0 | .. | 0 | .. |
| 7447 | 39 k | 0 | .. | 0 | .. | 7459 | 40 k | + | 25 | 0 | .. |
| 7448 | 40 k | 0 | .. | 0 | .. | 7460 | 40 k | + | 30 | 0 | .. |
| 7449 | 40 k | 0 | .. | 0 | .. | 7461 | 40 k | 0 | .. | 0 | .. |
| 7450 | 40 k | 0 | .. | 0 | .. | 7462 | 38 d | + | 34 | 0 | .. |
| 7451 | 40 k | 0 | .. | 0 | .. | 7463 | 40 k | + | 40 | 0 | .. |
| 7452 | 40 k | 0 | .. | 0 | .. | 7464 | 40 k | + | 21 | + | 11 |

Table 2.

Series A.

| Group No. | Addition | % of animals with exudative diathesis | % of animals with encephalomalacia | Total percentage of animals with E avitaminous symptoms |
|---|---|---|---|---|
| 1 | no | 100 | 38 | 100 |
| 2 | 0.1 mg d,l-α-tocopherol acetate | 45 | 0 | 45 |
| 3 | 0.2 - — — — | 35 | 0 | 35 |
| 4 | 0.4 - — — — | 0 | 0 | 0 |
| 5 | 0.8 - — — — | 0 | 0 | 0 |
| 6 | 1.6 - — — — | 0 | 0 | 0 |
| 7 | 3.2 - — — — | 0 | 0 | 0 |
| 8 | 250 - dried hips | 15 | 0 | 15 |
| 9 | 500 - — — | 5 | 0 | 5 |
| 10 | 1000 - — — | 5 | 0 | 5 |
| 11 | 2000 - — — | 0 | 0 | 0 |

Series B.

| Group No. | Addition | exudative diathesis | encephalomalacia | Total |
|---|---|---|---|---|
| 1 | no | 70 | 20 | 70 |
| 2 | 0.1 mg d,l-α-tocopherol acetate | 40 | 10 | 40 |
| 3 | 0.2 - — — — | 30 | 0 | 30 |
| 4 | 0.4 - — — — | 0 | 0 | 0 |
| 5 | 60 - dried hips | 30 | 20 | 50 |
| 6 | 120 - — — | 45 | 0 | 45 |
| 7 | 240 - — — | 55 | 0 | 55 |
| 8 | 60 - extract of dried hips | 50 | 30 | 70 |
| 9 | 120 - — - — — | 40 | 10 | 50 |
| 10 | 240 - — - — — | 28 | 11 | 39 |

Series C.

| Group No. | Addition | exudative diathesis | encephalomalacia | Total |
|---|---|---|---|---|
| 1 | no | 60 | 10 | 60 |
| 2 | 0.1 mg d,l-α-tocopherol acetate | 10 | 0 | 10 |
| 3 | 0.2 - — — — | 0 | 0 | 0 |
| 4 | 0.4 - — — — | 0 | 0 | 0 |
| 5 | .. distillate from alfalfa | 0 | 0 | 0 |
| 6 | .. undistillable from alfalfa | 75 | 8 | 75 |

Results and discussion. The results show that it is possible to use the chick for a preventive method of standardization of vitamin E by means of the vitamin's anti-exudative, resp. anti-encephalomalacia activity. The results indicate that the exudative symptom gives the most graduated relation between the dosage of the substance and the response; the evaluation of the experiments can therefore be carried out by means of the exudative symptom alone. The encephalomalacia symptom alone may be used under other circumstances, when diets richer in fat are given. In the present case, however, we preferred to calculate the total number of animals with symptoms of E avitaminosis.

When the basal diet without addition of vitamin E is fed (see, for instance, Table 3), the symptoms occur in 60—100 $^0/_0$ (an average of 80 $^0/_0$) of the animals. A comparison between the activity of two different sources of vitamin E can therefore be carried out in such a way that both substances are fed at the same time in different levels to different groups of chicks, and the doses are calculated which give symptoms by 40 $^0/_0$ of the animals. As is the case in similar standardizations, it is necessary to test, simultaneously with every standardization, the reference standard, d,l-α-tocopherol acetate, since the response to a given dose of the vitamin fluctuates, even if animals of the same stock and on the same diet are used. Table 3 shows the reproducibility of the chick's response to the same series of doses of the reference substance. These series are collected from the experiments in this and in the following parts of the paper.

The experiments show that the dosis of the reference substance which gives symptoms by 40 $^0/_0$ of the animals is about 0.1 mg per day.

Table 3.

| Series No. | Date of experiment | Duration of experiment days | mg of d,l-α-tocopherol acetate p. day | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 0.1 | 0.2 | 0.4 |
| | | | percentage of chicks with symptoms | | | |
| II A | 28/9-39 | about 35 | 100 | 45 | 35 | 0 |
| II B | 20/11-39 | — 40 | 70 | 40 | 30 | 0 |
| II C | 16/5-40 | — 39 | 60 | 60 | 5 | 0 |
| III B | 24/4-40 | — 35 | 90 | 35 | 25 | 0 |

In series B, we did not feed a dose of dried hips and extract of dried hips sufficient to yield 100 % protection; furthermore, the results for dried hips were unusually irregular, whereas extract of dried hips gave more regular results. If we try nevertheless to find the dosis showing symptoms by 40 % of the animals, the results of series B lead to an amount of about 240 mg of extract of dried hips per day, and B conferred with A gives about 240 mg of dried hips per day, in the case of d,l-α-tocopherol acetate the amount is 0.1 mg. This result is in rather good agreement with the chemical determinations described in Part I, which showed a tocopherol content of 0.124 mg in 240 mg hips and of 0.092 mg in the corresponding amount of extract, calculated as acetate; the values are of the same order of magnitude as those found in rat fertility tests in earlier experiments with hips. Even if the result is no exact standardization, it shows nevertheless that this method enables us to carry out vitamin E standardizations. If exact standardizations are to be carried out, the number of days until the symptoms occur should be taken into consideration. If the amount of vitamin E ingested is increased, the number of animals showing symptoms decreases and, besides, also the number of days till the appearence of the symptoms increases.

**Summary.**

The principles of a method of the biological standardization of vitamin E by means of its anti-exudative and anti-encephalomalacia activity are described. By means of this method it was tried to determine the vitamin E activity of hips.

## III. Comparison between the anti-exudative activity of α- und β-tocopherol.

The standardization method of Part II was applied in order to investigate whether the relation between the potency of α- and β-tocopherol in preventing exudative diathesis is the same as the relation between the potencies found in rat fertility tests.

Experimental. The experimental conditions with respect to keeping of the animals, diet, inspection for symptoms, autopsy etc. were the same as described in Part II. The results are summarized in tables with the same indications + and (+) for exudation symptoms as in Part II. The animals were inspected for encephalomalacia too, but no case showing this symptom could be found.

Two series of experiments, A and B, were carried out.

In series A, the effects of equal doses of α- and β-tocopherol acetates were compared. The tocopherol acetates were given in the form of tablets every day except Sunday. Each tablet contained 0.1 mg of the tocopherol acetate. The dose was 0.4 mg per animal per day until the weight had increased to 150 g. Thereafter, the dose increased by 50 % for every 50 g gain in weight over 100 g. The synthetic d,l-α-tocopherol acetate was supplied from F. Hoffmann-la Roche & Co. A.G., Basle. Synthetic β-tocopherol acetate was kindly put at our disposal by Prof. P. Karrer, Zürich.

In series B, the effects of varying doses of α- and β-tocopherol acetates were tested. The following doses were used: 0.05 — 0.1 — 0.2 — 0.4 mg of d,l-α- and 0.4 — 0.8 — 1.6 mg of d,l-β-tocopherol acetate per animal per day. The doses were kept constant through the whole period of experi-

Table 1.

Series A. Started 5/7-1939.

| Group 1 No addition of tocopherol acetate | | | | Group 2 Addition of d,l-α-tocopherol acetate | | | | Group 3 Addition of d,l-β-tocopherol acetate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Chick No. | Duration of experiment days | Exudate | days | Chick No. | Duration of experiment days | Exudate | days |
| 5563 | 6 d | 0 | .. | 5573 | 40 k | 0 | .. | 5583 | 19 k | + | 15 |
| 5564 | 5 d | 0 | .. | 5574 | 40 k | 0 | .. | 5584 | 17 d | 0 | .. |
| 5565 | 14 k | + | 13 | 5575 | 40 k | 0 | .. | 5585 | 15 k | + | 14 |
| 5566 | 29 d | 0 | .. | 5576 | 40 k | + | 7 | 5586 | 28 d | + | 27 |
| 5567 | 35 k | + | 31 | 5577 | 40 k | 0 | .. | 5587 | 5 d | 0 | .. |
| 5568 | 20 k | + | 19 | 5578 | 40 k | 0 | .. | 5588 | 11 d | 0 | .. |
| 5569 | 20 k | + | 17 | 5579 | 38 k | 0 | .. | 5589 | 27 k | + | 24 |
| 5570 | 15 k | + | 15 | 5580 | 9 d | 0 | .. | 5590 | 35 k | + | 35 |
| 5571 | 15 k | + | 14 | 5581 | 40 k | 0 | .. | 5591 | 19 d | 0 | .. |
| 5572 | 9 k | + | 7 | 5582 | 40 k | 0 | .. | 5592 | 20 k | + | 18 |

Series B. Started 24/4-1940.

| Group 1 No addition of tocopherol acetate | | | | Group 2 Addition of 0.05 mg d,l-α-tocopherol acetate | | | | Group 3 Addition of 0.1 mg d,l-α-tocopherol acetate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7401 | 34 k | + | 26 | 7391 | 34 k | 0 | .. | 7381 | 34 k | 0 | .. |
| 7402 | 34 k | + | 27 | 7392 | 34 k | + | 34 | 7382 | 34 k | 0 | .. |
| 7403 | 33 d | + | 12 | 7393 | 34 k | (+) | .. | 7383 | 34 k | + | 22 |
| 7404 | 34 k | + | 24 | 7394 | 34 k | + | 26 | 7384 | 34 k | 0 | .. |
| 7405 | 16 d | + | 16 | 7395 | 34 k | + | 15 | 7385 | 34 k | (+) | .. |
| 7406 | 34 k | + | 22 | 7396 | 34 k | 0 | .. | 7386 | 34 k | 0 | .. |
| 7407 | 35 k | + | 21 | 7397 | 35 k | + | 35 | 7387 | 35 k | + | 21 |
| 7408 | 35 k | + | 28 | 7398 | 35 k | + | 26 | 7388 | 29 d | + | 23 |
| 7409 | 24 d | + | 21 | 7399 | 35 k | + | 27 | 7389 | 35 k | 0 | .. |
| 7410 | 35 k | 0 | .. | 7400 | 32 d | (+) | .. | 7390 | 35 k | 0 | .. |

Table 1 (continued).

| Group 4 Addition of 0.2 mg d,l-α-tocopherol acetate | | | Group 5 Addition of 0.4 mg d,l-α-tocopherol acetate | | | Group 6 Addition of 0.4 mg d,l-β-tocopherol acetate | | |
|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate days | Chick No. | Duration of experiment days | Exudate days | Chick No. | Duration of experiment days | Exudate days |
| 7371 | 34 k | 0 .. | 7361 | 34 k | 0 .. | 7431 | 34 k | + 34 |
| 7372 | 34 k | 0 .. | 7362 | 34 k | 0 .. | 7432 | 34 k | + 34 |
| 7373 | 34 k | (+) | 7363 | 34 k | 0 .. | 7433 | 34 k | + 22 |
| 7374 | 34 k | 0 .. | 7364 | 34 k | 0 .. | 7434 | 34 k | + 33 |
| 7375 | 34 k | 0 .. | 7365 | 34 k | 0 .. | 7435 | 34 k | 0 .. |
| 7376 | 34 k | 0 .. | 7366 | 22 d | 0 .. | 7436 | 34 k | + 34 |
| 7377 | 35 k | + 35 | 7367 | 35 k | 0 .. | 7437 | 35 k | + 33 |
| 7378 | 35 k | 0 .. | 7368 | 35 k | 0 .. | 7438 | 35 k | + 13 |
| 7379 | 35 k | 0 .. | 7369 | 35 k | 0 .. | 7439 | 35 k | + 28 |
| 7380 | 35 k | + 35 | 7390 | 35 k | 0 .. | 7440 | 35 k | + 33 |
| Group 7 Addition of 0.8 mg d,l-β-tocopherol acetate | | | Group 8 Addition of 1.6 mg d,l-β-tocopherol acetate | | | | | |
| 7421 | 16 d | 0 .. | 7411 | 34 k | + 12 | | | |
| 7422 | 34 k | + 26 | 7412 | 16 d | 0 .. | | | |
| 7423 | 34 k | + 20 | 7413 | 34 k | + 26 | | | |
| 7424 | 34 k | + 22 | 7414 | 34 k | + 21 | | | |
| 7425 | 34 k | 0 .. | 7415 | 34 k | 0 .. | | | |
| 7426 | 34 k | + 33 | 7416 | 34 k | + 23 | | | |
| 7427 | 35 k | + 21 | 7417 | 35 k | + 33 | | | |
| 7428 | 25 d | + 12 | 7418 | 35 k | + 21 | | | |
| 7429 | 35 k | + 20 | 7419 | 35 k | 0 .. | | | |
| 7430 | 35 k | + 33 | 7420 | 32 d | + 17 | | | |

ment. The β-tocopherol acetate applied in this experiment was prepared in our laboratory following the method of Karrer and co-workers[1]. The phytol used in the synthesis was kindly supplied by F. Hoffmann-la Roche & Co. A.G. and Chemische Fabrik vormals Sandoz, Basle. The β-tocopherol was acetylated and again purified by cromatographic

[1] P. Karrer, H. Koenig, B. H. Ringier and H. Salomon, Helv. chim. acta **22**, 1139 (1939).

Table 2.

Series A.

| Addition | % of animals with exudate |
|---|---|
| no | 88 |
| d,l-α-tocopherol acetate | 10 |
| d,l-β-tocopherol acetate | 67 |

Series B.

| Addition | % of animals with exudate |
|---|---|
| no | 90 |
| 0.05 mg d,l-α-tocopherol acetate | 70 |
| 0.1 mg — | 35 |
| 0.2 mg — | 25 |
| 0.4 mg — | 0 |
| 0.4 mg d,l-β-tocopherol acetate | 90 |
| 0.8 mg — | 80 |
| 1.6 mg — | 70 |

Table 3.

| Addition | Weight g | | | | | |
|---|---|---|---|---|---|---|
| | 24/4 | 1/5 | 8/5 | 15/5 | 22/5 | 28/5 |
| no | 96 | 122 | 147 | 191 | 229 | 229 |
| 0.05 mg d,l-α-tocopherol acetate | 91 | 110 | 133 | 172 | 222 | 241 |
| 0.1 mg — | 91 | 112 | 132 | 170 | 191 | 205 |
| 0.2 mg — | 89 | 111 | 126 | 172 | 210 | 234 |
| 0.4 mg — | 89 | 106 | 128 | 163 | 193 | 210 |
| 0.4 mg d,l-β-tocopherol acetate | 100 | 120 | 132 | 177 | 211 | 241 |
| 0.8 mg — | 91 | 115 | 136 | 181 | 200 | 215 |
| 1.6 mg — | 89 | 118 | 144 | 202 | 231 | 251 |

absorption on aluminium oxide. With respect to its absorption spectrum it agreed with the spectrum of neo-tocopherol as found by Karrer[1]. Furthermore, its equivalent weight, determined by reduction of $FeCl_3$-dipyridyl after Emmerie and Engel, agreed with its formula.

The animals were weighed once a week. In series A, the animals were killed as soon as they showed exudates;

[1] P. Karrer, H. Salomon and H. Fritzsche, Helv. chim. acta 21, 309 (1938).

in series B, however, they were kept alive until the 34th day. A summary of the weights of the groups of this series is given in Table 3.

Results and discussion. Table 2, series A, clearly shows that there is a great difference in the effectiveness of α- and β-tocopherol acetates in preventing the exudative diathesis in chicks.

In series B, it was attempted to evaluate the relative activity of the two compounds by testing the effects of a series of doses of each of them. From Table 2, series B, it appears, however, that the potency of β-tocopherol acetate is so small that even a dose which was four times as great as the completely protecting dose of the α-compound did not exert any protective effect at all.

Since a dose of β-tocopherol acetate sufficient to protect against the symptom has not been tested, it is impossible from the results obtained in these experiments to determine the relative anti-exudative potency of the components. The results, however, suggest that the potency of the β-compound is less than 5 % of that of the α-compound.

Previous experiments carried out in several laboratories have shown that α-tocopherol has a potency in preventing rat sterility of 3—4 times that of β-tocopherol. We compared the potencies of our preparations used in series B by means of this test too. This experiment was kindly carried out by cand. pharm. Kjerbye-Nielsen, A/S Medicinalco, following the method used in this laboratory. 0.7 mg of d,l-α-tocopherol acetate gave 65 % protection; 4 mg of d,l-β-tocopherol acetate gave 100 % protection, 2 mg and 1 mg gave 0 % protection, which is in accordance with the earlier experiments.

In the chick experiment, however, it was found that 0.05 mg of d,l-α-tocopherol acetate was more potent than 1.6 mg of d,l-β-tocopherol acetate, i. e. that α-tocopherol is more than 30 times as potent as β-tocopherol. It is, therefore, possible that the relative potency of the E vitamins in preventing the most characteristic symptom of E avitaminosis in chicks is not the same as the potency of the same compounds in preventing rat sterility.

If this statement holds, it would be an analogy of the properties of the D vitamins, where the antirachitic potency of the vitamins $D_2$ and $D_3$ is not the same when tested on rats or on chicks.

It would be of great interest to reinvestigate this question by using a greater number of chicks and higher doses of β-tocopherol than applied in this experiment.

It would also be of interest to investigate the anti-encephalomalacia-activity of β-tocopherol acetate in relation to that of the α-tocopherol acetate. This would afford a diet much richer in fat than the diet used in this experiment in which the presence of 5 $^0/_0$ cod liver oil was not sufficient to develop cases of encephalomalacia.

From Table 3 it appears that d,l-α-tocopherol acetate has no influence on the growth of chicks, when this diet is used. In earlier experiments, however, when other diets were used, we have seen a significant influence of tocopherol upon the growth of the chicks. It would be of interest to examine this question further and especially to investigate the influence of vitamin E on the growth of chicks being fed with a diet rich in fat.

**Summary.**

Whilst d,l-α-tocopherol acetate is 3—4 times as active as d,l-β-tocopherol acetate in preventing rat sterility, it was found to be at least 30 times as active as the β-compound in preventing exudative diathesis in chicks.

## IV. The influence of fat on the development of encephalomalacia and exudative diathesis in chicks.

In accordance with the experience of Pappenheimer and Goettsch and their co-workers[1], we observed that the encephalomalacia occurs in a greater percentage of the experimental animals when the diet contains much fat, a fact which is also in agreement with the still earlier experience that the most commonly known symptom of E avitaminosis, viz. the sterility, is particularly easy to develop by means of a fat rich diet.

As regards the exudative diathesis, our earlier experiments did not reveal any large difference in the percentage of animals afflicted with this symptom, dependent on whether the diet contained extra fat or not.

Recently, we had an opportunity of investigating the influence of fat on both symptoms on a larger scale. The results of these experiments are reported in the following.

Experimental. Experimental conditions with respect to housing of the animals, inspection for symptoms etc. were the same as described in Part II. As basal diet we used diet 538 (cfr. Part II). Other diets were prepared by addition of lard resp. cod liver oil to diet 538.

Vitamins A and D were given in the form of a mixture

[1] A. M. Pappenheimer, Marianne Goettsch and E. Jungherr, Storrs Agricultural Experiment Station Bulletin **229** (Jan. 1939).

Table 1.

| Series A Started 8/1-1940 | | | | | | Series B Started 9/3-1940 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| **Group 1: Diet 538 without addition of lard** | | | | | | | | | | | |
| 7201 | 49 k | 0 | .. | + | 47 | 7241 | 49 k | + | 31 | 0 | .. |
| 7202 | 49 k | + | 39 | 0 | .. | 7242 | 49 k | 0 | .. | 0 | .. |
| 7203 | 49 k | (+) | .. | 0 | .. | 7243 | 46 k | 0 | .. | 0 | .. |
| 7204 | 50 k | 0 | .. | 0 | .. | 7244 | 49 k | 0 | .. | 0 | .. |
| 7205 | 50 k | + | 35 | 0 | .. | 7245 | 49 k | 0 | .. | 0 | .. |
| 7206 | 50 k | + | 50 | 0 | .. | 7246 | 51 k | 0 | .. | 0 | .. |
| 7207 | 4 d | 0 | .. | 0 | .. | 7247 | 51 k | (+) | .. | 0 | .. |
| 7208 | 49 k | 0 | .. | 0 | .. | 7248 | 51 k | 0 | .. | 0 | .. |
| 7209 | 49 k | + | 49 | 0 | .. | 7249 | 51 k | 0 | .. | 0 | .. |
| 7210 | 49 k | 0 | .. | 0 | .. | 7250 | 52 k | 0 | .. | + | 30 |
| 7211 | 49 k | 0 | .. | 0 | .. | 7251 | 2 d | 0 | .. | 0 | .. |
| 7212 | 36 d | 0 | .. | 0 | .. | 7252 | 52 k | 0 | .. | 0 | .. |
| **Group 2: Diet 538 + 1 % of lard.** | | | | | | | | | | | |
| 7143 | 49 k | + | 49 | 0 | .. | 7253 | 49 k | 0 | .. | 0 | .. |
| 7144 | 49 k | 0 | .. | 0 | .. | 7254 | 49 k | 0 | .. | 0 | .. |
| 7145 | 49 k | 0 | .. | 0 | .. | 7255 | 49 k | 0 | .. | 0 | .. |
| 7146 | 49 k | 0 | .. | 0 | .. | 7256 | 49 k | 0 | .. | 0 | .. |
| 7147 | 50 k | + | 44 | 0 | .. | 7257 | 49 k | 0 | .. | 0 | .. |
| 7148 | 49 k | 0 | .. | 0 | .. | 7258 | 51 k | 0 | .. | 0 | .. |
| 7149 | 50 k | (+) | .. | 0 | .. | 7259 | 41 d | + | 16 | 0 | .. |
| 7150 | 50 k | (+) | .. | 0 | .. | 7260 | 51 k | + | 31 | 0 | .. |
| 7151 | 49 k | 0 | .. | + | 49 | 7261 | 52 k | 0 | .. | 0 | .. |
| 7152 | 49 k | 0 | .. | 0 | .. | 7262 | 52 k | 0 | .. | 0 | .. |
| 7153 | 49 k | 0 | .. | 0 | .. | 7263 | 52 k | 0 | .. | 0 | .. |
| 7154 | 49 k | 0 | .. | 0 | .. | 7264 | 22 d | 0 | .. | 0 | .. |
| **Group 3: Diet 538 + 3 % of lard.** | | | | | | | | | | | |
| 7155 | 49 k | 0 | .. | 0 | .. | 7265 | 49 k | 0 | .. | 0 | .. |
| 7156 | 49 k | 0 | .. | 0 | .. | 7266 | 49 k | 0 | .. | 0 | .. |
| 7157 | 44 d | + | 44 | 0 | .. | 7267 | 49 k | 0 | .. | 0 | .. |
| 7158 | 49 k | 0 | .. | 0 | .. | 7268 | 49 k | + | 37 | 0 | .. |
| 7159 | 7 d | 0 | .. | 0 | .. | 7269 | 32 d | 0 | .. | 0 | .. |
| 7160 | 50 k | 0 | .. | 0 | .. | 7270 | 51 k | 0 | .. | 0 | .. |

Table 1 (continued).

| Series A Started 8/1-1940 | | | | | | Series B Started 9/3-1940 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| **Group 3 (continued).** | | | | | | | | | | | |
| 7161 | 50 k | 0 | .. | 0 | .. | 7271 | 51 k | + | 49 | 0 | .. |
| 7162 | 49 k | 0 | .. | 0 | .. | 7272 | 51 k | + | 48 | 0 | .. |
| 7163 | 49 k | 0 | .. | 0 | .. | 7273 | 51 k | + | 48 | 0 | .. |
| 7164 | 49 k | 0 | .. | 0 | .. | 7274 | 52 k | (+) | .. | 0 | .. |
| 7165 | 49 k | 0 | .. | + | 49 | 7275 | 52 k | 0 | .. | 0 | .. |
| 7166 | 49 k | + | 39 | 0 | .. | 7276 | 52 k | 0 | .. | 0 | .. |
| **Group 4: Diet 538 + 9 % of lard.** | | | | | | | | | | | |
| 7167 | 49 k | 0 | .. | 0 | .. | 7277 | 49 k | 0 | .. | 0 | .. |
| 7168 | 49 k | 0 | .. | 0 | .. | 7278 | 49 k | + | 42 | 0 | .. |
| 7169 | 37 d | 0 | .. | 0 | .. | 7279 | 49 k | + | 49 | 0 | .. |
| 7170 | 49 k | 0 | .. | 0 | .. | 7280 | 49 k | 0 | .. | 0 | .. |
| 7171 | 45 d | + | 36 | 0 | .. | 7281 | 51 k | 0 | .. | + | 49 |
| 7172 | 43 d | + | 15 | 0 | .. | 7282 | 51 k | + | 45 | 0 | .. |
| 7173 | 50 k | (+) | .. | 0 | .. | 7283 | 51 k | 0 | .. | 0 | .. |
| 7174 | 49 k | + | 41 | 0 | .. | 7284 | 51 k | 0 | .. | 0 | .. |
| 7175 | 49 k | + | 44 | 0 | .. | 7285 | 52 k | + | 46 | 0 | .. |
| 7176 | 49 k | 0 | .. | 0 | .. | 7286 | 52 k | 0 | .. | 0 | .. |
| 7177 | 49 k | 0 | .. | 0 | .. | 7287 | 52 k | + | 52 | 0 | .. |
| 7178 | 49 k | 0 | .. | 0 | .. | 7288 | 52 k | + | 52 | 0 | .. |
| **Group 5: Diet 538 + 27 % of lard.** | | | | | | | | | | | |
| 7179 | 28 d | + | 22 | 0 | .. | 7289 | 49 k | + | 34 | + | 44 |
| 7180 | 47 d | + | 40 | 0 | .. | 7290 | 49 k | 0 | .. | + | 45 |
| 7181 | 26 d | (+) | .. | + | 21 | 7291 | 49 k | + | 21 | 0 | .. |
| 7182 | 49 k | 0 | .. | 0 | .. | 7292 | 49 k | + | 26 | + | 45 |
| 7183 | 49 k | + | 41 | + | 46 | 7293 | 23 d | + | 11 | + | 22 |
| 7184 | 49 k | + | 43 | 0 | .. | 7294 | 51 k | + | 47 | + | 22 |
| 7185 | 39 d | + | 37 | 0 | .. | 7295 | 51 k | 0 | .. | + | 22 |
| 7186 | 43 k | (+) | .. | 0 | .. | 7296 | 51 k | 0 | .. | 0 | .. |
| 7187 | 48 k | (+) | .. | + | 30 | 7297 | 51 k | 0 | .. | + | 42 |
| 7188 | 48 k | 0 | .. | 0 | .. | 7298 | 52 k | + | 52 | 0 | .. |
| 7189 | 43 d | + | 32 | + | 20 | 7299 | 52 k | 0 | .. | 0 | .. |
| 7190 | 24 d | 0 | .. | + | 17 | 7300 | 52 k | + | 46 | 0 | .. |

Table 2.

| % of lard | Series A | | | Series B | | |
|---|---|---|---|---|---|---|
| | % of animals | | | % of animals | | |
| | with exudation | with encephalo-malacia | surviving the 49th day | with exudation | with encephalo-malacia | surviving the 49th day |
| 0 | 41 | 9 | 91 | 14 | 9 | 100 |
| 1 | 25 | 8 | 100 | 17 | 0 | 83 |
| 3 | 8 | 9 | 100 | 38 | 0 | 92 |
| 9 | 38 | 0 | 83 | 50 | 8 | 100 |
| 27 | 63 | 42 | 50 | 58 | 58 | 92 |

Table 3.

Series A.

| % of lard | Weight g | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8/1 | 13/1 | 20/1 | 27/1 | 3/2 | 10/2 | 17/2 | 24/2 |
| 0 | 95 | 106 | 135 | 170 | 208 | 236 | 264 | 293 |
| 1 | 100 | 112 | 142 | 176 | 219 | 259 | 291 | 326 |
| 3 | 101 | 109 | 139 | 179 | 207 | 242 | 264 | 284 |
| 9 | 101 | 112 | 136 | 163 | 182 | 200 | 233 | 228 |
| 27 | 96 | 102 | 117 | 139 | 145 | 165 | 180 | 189 |
| 1 mg d, l-α-tocopherol acetate. | 95 | 104 | 138 | 177 | 207 | 244 | 265 | 301 |

Series B.

| % of lard | Weight g | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 9/3 | 16/3 | 23/3 | 30/3 | 6/4 | 13/4 | 20/4 | 27/4 |
| 0 | 119 | 117 | 151 | 193 | 247 | 274 | 295 | 340 |
| 1 | 112 | 132 | 164 | 207 | 269 | 294 | 307 | 357 |
| 3 | 114 | 136 | 176 | 242 | 300 | 336 | 369 | 417 |
| 9 | 118 | 134 | 163 | 197 | 247 | 281 | 323 | 356 |
| 27 | 110 | 118 | 134 | 151 | 160 | 183 | 193 | 205 |

Table 4.

| Group 1<br>Diet 538 + 5 % of lard | | | | | Group 2<br>Diet 538 + 5 % of cod liver oil | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Started 16/5—1940 | | | | | | | | | |
| Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days | Chick No. | Duration of experiment days | Exudate | days | Encephalomalacia | days |
| 9070 | 39 k | 0 | .. | 0 | .. | 9080 | 39 d | + | 31 | 0 | .. |
| 9071 | 39 k | 0 | .. | 0 | .. | 9081 | 34 d | + | 27 | 0 | .. |
| 9072 | 39 k | 0 | .. | 0 | .. | 9082 | 39 k | 0 | .. | 0 | .. |
| 9073 | 39 k | 0 | .. | + | 14 | 9083 | 39 k | 0 | .. | 0 | .. |
| 9074 | 34 d | + | 30 | 0 | .. | 9084 | 39 k | + | 39 | 0 | .. |
| 9075 | 40 k | + | 36 | 0 | .. | 9085 | 39 k | + | 27 | + | 33 |
| 9076 | 35 d | + | 30 | 0 | .. | 9086 | 36 k | + | 29 | 0 | .. |
| 9077 | 40 k | + | 33 | 0 | .. | 9087 | 30 d | + | 16 | 0 | .. |
| 9078 | 40 k | + | 24 | 0 | .. | 9088 | 40 k | 0 | .. | 0 | .. |
| 9079 | 40 k | 0 | .. | 0 | .. | 9089 | 40 k | 0 | .. | 0 | .. |

Summary

| | % of animals | |
|---|---|---|
| | with exudative diathesis | with encephalomalacia |
| Diet 538 with 5 % of lard .......... | 50 | 10 |
| Diet 538 with 5 % of cod liver oil .. | 60 | 10 |

of 1 part of Vogan + 2 parts of Vigantol (Bayer); 0.05 cc three times a week.

Vitamin K was supplied as tablets containing 0.005 mg of pure $K_1$ per tablet. One tablet was given 6 days of the week. For every 50 g gain in weight, the dose was increased by 50 %.

Two series of experiments, A and B, were carried out with the same diets and under the same experimental conditions; they were started with an interval of 2 months.

The results are summarized in Tables 1 and 2 which are arranged as in Part II making use of the same indications.

As already mentioned, the animals were weighed once a week. Even if the ratio between protein and total calories was not kept constant from one diet to another, it may, perhaps, be of interest to give a summary of the weights. This is done in Table 3.

In series A, the group without lard was accompanied by a corresponding group in which 1 mg of d,l-α-tocopherol acetate in the form of a tablet was given to every animal every day except Sunday. This group is not included in Table 1 because all the animals were completely protected against both symptoms. The mean weights of the animals of this group are to be seen in Table 3.

Table 4 shows the results from two experiments carried out simultaneously, group 1 with 5 $^0/_0$ of lard added to the diet, and group 2 with 5 $^0/_0$ of cod liver oil; a summary of these experiments is given below the table. Group 1 received vitamins A and D in the form of 0.05 cc of a mixture of 5 cc Vogan and 10 cc Vigantol three times a week.

Results and discussion. Both series A and B show that not only encephalomalacia but also exudative diathesis is markedly favoured by the presence of much fat in the diet. Our previous view, viz. that large amounts of fat do not favour the occurrence of exudative diathesis, could therefore not be maintained. Table 2 as well as the tables of the papers published on the foregoing pages (22 and 28) indicate, however, that exudative diathesis is the prevailing symptom of the diets poor in fat. In the groups with up to 9 per cent of lard the cases of exudates were 6 times as numerous as those of encephalomalacia, while in the group with 27 per cent of lard almost as many cases of

encephalomalacia as of exudative diathesis were found (comp. Table 2).

In certain previous experiments with animals living on a diet containing 5 % of cod liver oil, we worked with groups of chicks on which encephalomalacia was easily developed. On the other hand, no case of encephalomalacia was noticed on the chicks of Part III of the present series of papers; these chicks, too, were fed on a diet containing 5 % of cod liver oil. In order to check whether cod liver oil had a greater influence on the development of encephalomalacia and exudative diathesis than lard, we fed two groups, simultaneously, with diet 538 + 5 % of lard and 5 % of cod liver oil, respectively (Table 4). These two groups behaved rather similar with respect to both symptoms and, moreover, encephalomalacia occurred only in a few cases. Thus, cod liver oil does apparently not favour the symptoms more than lard does. There seems to be a still unexplained variability in the easiness with which the symptoms are developed. It is worth while noting that, in experiments with the encephalomalacia producing diet 108 of PAPPENHEIMER and GOETTSCH[1], exudates appear only very rarely. It would be of interest to investigate whether small amounts of tocopherol, as may be present in the not extracted constituents of diet 108, added to the fat rich diets prevent exudates without materially suppressing the encephalomalacia.

The presence of 1—3 % of lard in the diet does not favour the occurrence of exudative diathesis or encephalomalacia. The results found on series A might, if anything, suggest that 1—3 % of lard in the diet were favourable to suppressing the symptom as well as, perhaps, to increase

[1] A. M. PAPPENHEIMER and MARIANNE GOETTSCH, J. Exp. Med. **53**, 11 (1931).

the time of survival of the chicks. In order to settle this question definitely it will be advisable to carry out further experiments with greater groups of chicks.

In Table 3, we publish the weights of the chicks which may be of some interest although it is not easy to interpret the results on account of the fact that the ratio protein: total calories of the diet was not kept constant. It is clear from this table that the presence of 1—3 % of lard in the diet favours the growth of the chicks.

A great number of experiments by other investigators deal with the influence of the fat content of diets low in vitamin E, upon the occurence of sterility in rats. The view generally held is this that lard contains substances which exert a destructive effect on vitamin E[1]. Our present experiments do not contribute to the solution of the question of an antagonistic action of fat against vitamin E. However, the experiments described above and in earlier papers, which show the effect of small doses of tocopherol in diets rich in fat, may best be explained by the view that the destruction of the vitamin by substances contained in fat may be the decisive factor for an explanation of many of the experiences with vitamin E; on the other hand, there may possibly exist still further relations between the amount of fat in the diet and the requirement of vitamin E.

This important question may perhaps be studied successfully by means of chicks, since it could be proved that all three manifestations of E-avitaminosis, viz. sterility, encephalomalacia and exudative diathesis, are influenced by fat and that these chick symptoms are developed much more rapidly than the rat sterility.

[1] H. A. Mattill, American Medical Association: The vitamins p. 575 (1939).

**Summary.**

The occurrence of both encephalomalacia and exudative diathesis was found to be favoured by the presence of much fat in the diet; the exudative diathesis was found, however, to be the prevailing symptom in chicks living on diets low in fat. Cod liver oil did not increase the number of chicks with symptoms more than lard. The presence of 1—3 per cent of lard in vitamin E-free diets seems to be favourable with respect to protection against E-avitaminous symptoms as well as to the growth of the chicks.

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Since this paper went to press, symptoms resembling exudative diathesis in chicks have been described by H. R. Bird, T. G. Culton and O. L. Kline in J. Nutrition, **19**, 15 (1940). Earlier the symptom was described by Pappenheimer, Goettsch and Jungherr, (loc. cit. p. 30).

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