LXXIX. THE QUANTITATIVE ESTIMATION OF THE FAT-SOLUBLE FACTOR.

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The technique hitherto employed in the estimation of the fat-soluble factor has been of a more or less qualitative nature. The substance to be tested has usually been incorporated in a basal diet free from the factor and the potency determined by the tendency of the animal to grow. As the work on the fat-soluble factor progressed it became evident that a quantitative method was desirable for the estimation of the principle in natural products as well as in various fractions obtained in chemical manipulations. No chemical method for establishing the presence of the accessory factors is at present available and we therefore had recourse to the study of suitable conditions under which results of a quantitative nature could be obtained with the biological method now in use.

In the case of the antiscorbutic and the antineuritic factors, quantitative methods have been employed in this Institute for some time. The technique used in the estimation of these factors lends itself more to quantitative work, since the animals employed for the purpose, namely guinea pigs, monkeys, and pigeons can be fed by hand without difficulty, thus affording the opportunity of administering definite quantities of the tested material. Moreover, since the dietetic deficiency in these cases manifests itself by symptoms of well-defined diseases such as scurvy and polyneuritis, the minimum dose necessary to prevent the onset of the disease can be determined and used as a standard of comparison. The deficiency of the fat-soluble factor in the diet of rats, however, does not manifest itself by clinical symptoms with such regularity as could be utilised for comparative purposes. It is well known that the occurrence of keratomalacia in rats subsisting on a fat-soluble-free diet is irregular and consequently it cannot be employed as a standard. We were, therefore, obliged to restrict ourselves to observations on the growth. This, although somewhat vitiated by individual variations, has nevertheless the advantage of being a regular manifestation; rats which have ceased to grow owing to a deficiency of the fat-soluble factor seldom fail to respond to the addition of the active principle.

We do not intend to describe all the numerous experiments which were performed in connection with this inquiry and shall only discuss the conditions

under which we find that quantitative results can be obtained in the estimation of the fat-soluble factor.

At the outset it is essential to point out that a healthy stock of rats must be employed in order to ensure good results. It is evident that any restriction in growth produced by factors other than the deficiency of the fat-soluble factor will lead to erroneous conclusions. Animals weighing 50–60 g. are placed on the usual basal diet, and, if the diet is properly purified, the majority of them show only little growth during the first 10–15 days after which time they cease to grow altogether. Some vigorous rats in spite of the deficiency grow to a marked extent during the first few weeks. Such animals which form only a small percentage of the total number employed are useless for quantitative work, although they can be employed for the ordinary detection tests of the fat-soluble factor. The animals are kept for three to four weeks on the basal diet before the actual testing commences. By the end of this period the rats have not been gaining in weight for some time and any addition of the factor produces a definite response in increase of weight. No rats which have at this stage attained a weight higher than 70 g. are suitable for testing purposes.

In estimating the potency of a substance it is administered in known quantities separately without being incorporated in the diet, the mode of administration varying with the character of the substance to be tested. Our work was confined mainly to oils which are liquid at ordinary temperature such as fish oils, and fats which are solid at ordinary temperature such as butter, margarine, etc.

In the case of liquid oils the following procedure is adopted. The oil is delivered in drops of known weight from a pipette into a small pellet of the basal diet containing a depression and is then covered with a little more of the powdered diet. These pellets are given with the aid of a spatula to the animals which have ceased growing. The rats soon become used to take the dose without difficulty and consume it at once. The daily dose of the basal ration can then be served out. The desired quantity of oil can also be delivered directly into the mouth of the animal. This procedure although quicker requires a certain skill and is unsuitable for routine work when delegated to laboratory assistants. In the case of very potent oils such as cod liver oil, when less than a drop is required, the oil is previously diluted to the desired strength with an inactive oil such as oxidised olive oil and a drop of the diluted oil is employed.

Solid fats are previously melted at a low temperature and the requisite number of drops of known weight are allowed to solidify and are fed with a spatula before the animals receive the basal diet. This procedure is decidedly quicker than weighing each dose separately.

As a standard of comparison we take the least dose which induces definite growth after the animals have ceased to grow owing to the deficient basal diet. This of course has the disadvantage of being a relative standard which might vary with the individual observer, but our experience leads us to believe that it is the only reliable standard under the circumstances and that it is

possible to obtain fairly accurate results with it. Our original intention was to take the minimum dose required to produce normal growth but it was found that the magnitude of such a dose could not be sharply defined from the weight curves. When the dose was gradually reduced a point was reached at

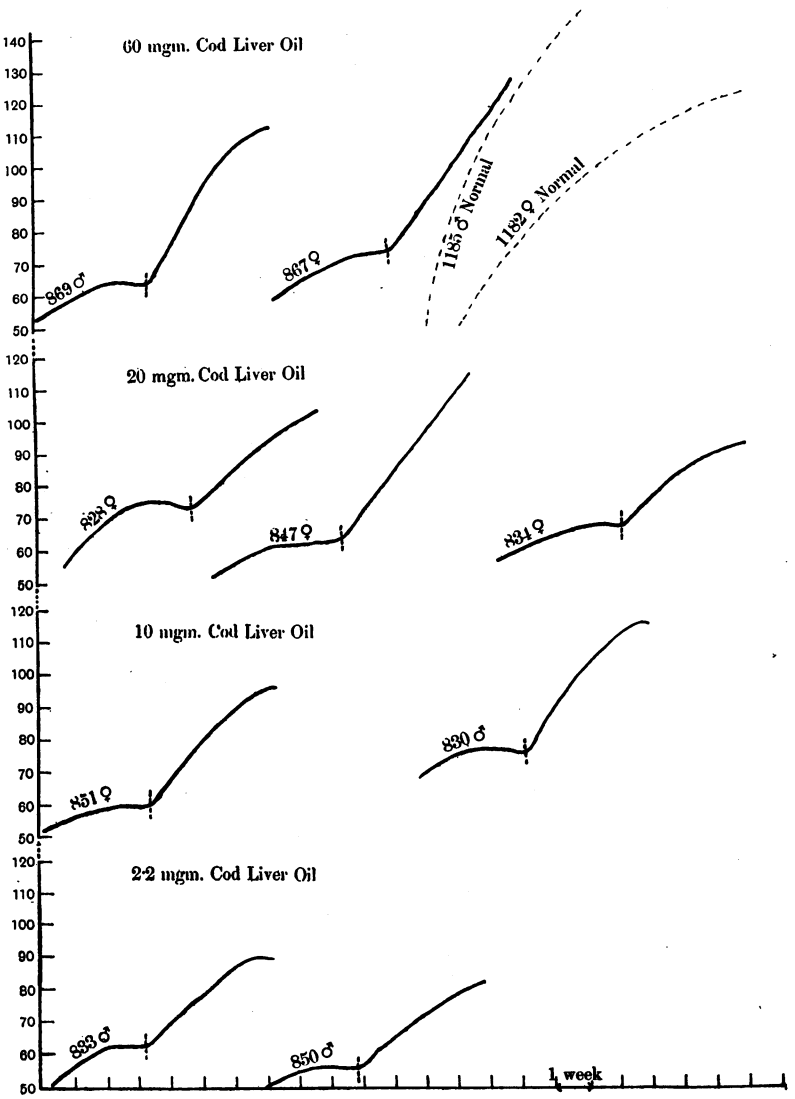

Fig. 1.

which definite growth was still obtained, but any further reduction made the dose inadequate to promote appreciable growth in the majority of the animals. This is illustrated by Figs. 1 and 2 which represent the growth curves of rats which received doses of various magnitudes of a very potent cod-liver oil.

Normal growth was obtained with about 20 mgms. of the oil and the diminution in the rate of growth brought about by reducing the dose was gradual. With 1·7 mgm., *i.e.* 1/12 of a drop (obtained by diluting the original oil), definite growth was still obtained. On reducing the dose to 1·4 mgm. no appreciable growth was recorded. The line of demarcation at this stage is well marked. We therefore take the minimum dose which induces definite growth for four weeks as our standard. Quite consistent results can thus be obtained and the relative potency of various active substances can be established with fair accuracy. This method has also been utilised lately in another

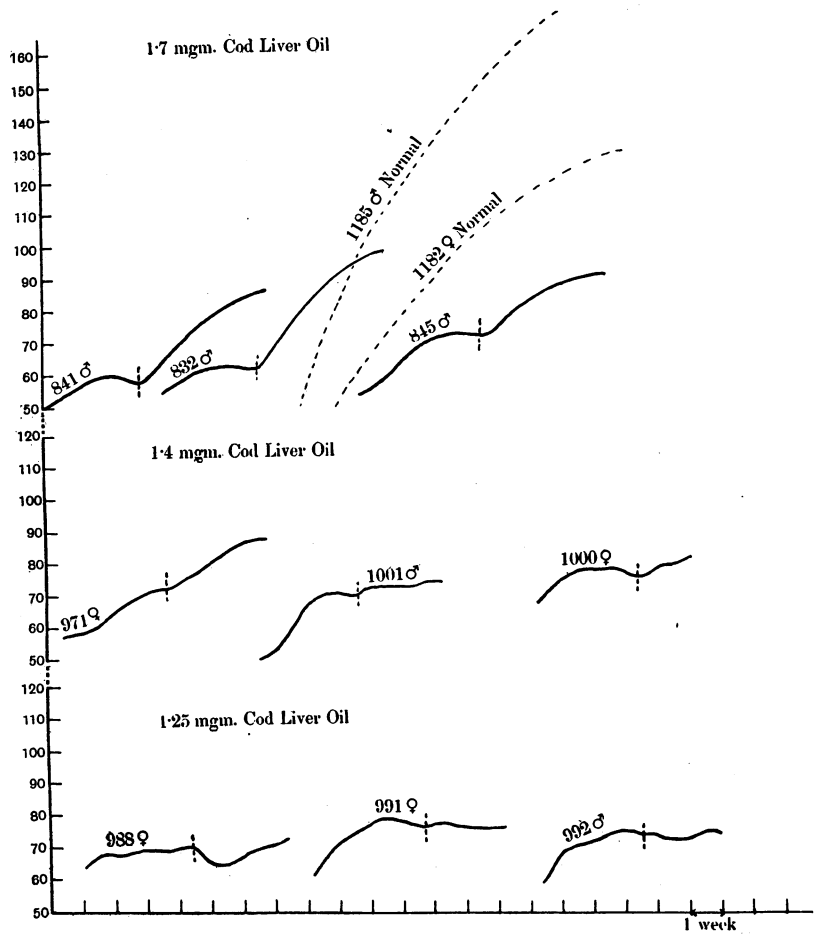

Fig. 2.

laboratory in connection with a joint investigation and equally consistent results were obtained there independently.

In the course of this investigation we had the opportunity of observing the exceptional potency of cod-liver oil. We examined various oils and found that the minimum dose varied from 1·7–5 mgms. The butters which were tested,

on the other hand, were found to be in comparison very much less potent. As will be seen from Fig. 3, the weight curves of animals fed on one of our samples of butter, the minimum dose lies between 200 and 400 mgms. Some butters were even less potent. We [1921] have already expressed the opinion elsewhere that the striking therapeutic value of cod-liver oil is no doubt due to this high vitamin content. In this connection it is interesting to point out that the superiority of cod-liver oil over butter in the treatment of rickets

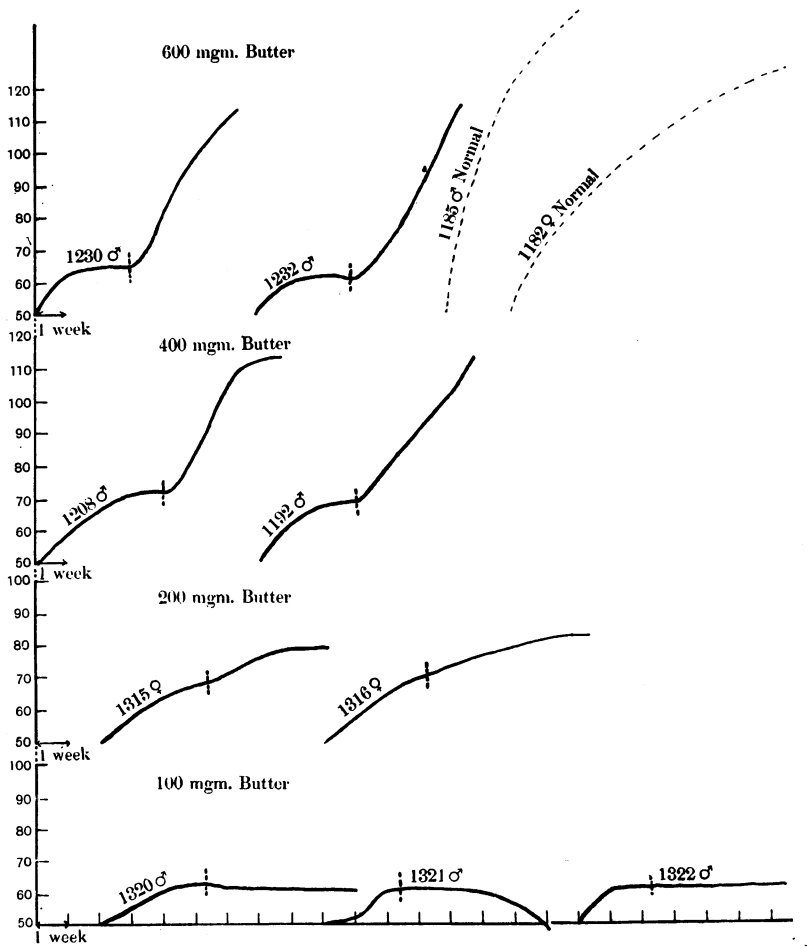

Fig. 3.

was not unknown to some clinicians. Trousseau [1872] in his lecture on rickets, discussing the value of cod-liver oil in the treatment of the disease, refers to butter as follows: "there is one substitute (for cod-liver oil), *butter*, within the reach of all, from which excellent results are obtained; but to obtain such results it is necessary that a large quantity, sixty grams, at least, be taken in the 24 hours." We consider that the difference in the activity of the two

substances is due not to the presence of two different principles as has recently been suggested but to the higher content of the fat-soluble factor in one of them.

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Summary.

A quantitative method for the estimation of the fat-soluble factor is described.

The exceptionally high potency of cod-liver oil is demonstrated.

REFERENCES.

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