Summary.

1. A technique for the investigation of the hæmolytic action of chemical substances is described.

2. The relation between the time taken by a given quantity of hæmolytic substance, and the temperature at which it acts, is expressed by a hyperbola.

3. Equations are given expressing the relation between the constants of such a hyperbola and the quantity of hæmolytic substance to which the hyperbola applies.

4. Certain general relations, which have been found to hold for all substances examined in connection with this research, are pointed out.

5. A comparison between experimental and calculated results is given.

Observations on the Effects of Fat Excess on the Growth and Metamorphosis of Tadpoles.

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Previous experimentation* has shown that in certain circumstances the presence of excessive amounts of fat in the food of animals may be harmful. Thus, an excess of butter in association with a dietary of autoclaved rice hastens the death of both pigeons and monkeys, and gives rise to changes in the internal organs more pronounced than those resulting from an autoclaved rice dietary alone. Again, an excess of butter in association with a dietary of mixed grains and peas causes enlargement, with hyperplasia and vesicular budding, of the thyroid gland in pigeons, identical with that characteristic of Graves' disease.† This enlargement of the thyroid gland is associated with a reduction in size of the adrenal glands. If, however, fresh onions be added to the dietary of mixed grains and butter both the incidence of the thyroid enlargement and the intensity of the hyperplasia are reduced; while the associated diminution in size of the adrenal glands is not so marked.

This observation as to the effect of an otherwise adequate food containing an excess of butter in producing thyroid hyperplasia of the Graves' disease

* McCarrison, R., 'Ind. Jour. Med. Res.,' vol. 6 (4), p. 550, and vol. 7 (2), p. 308 (1919).
† McCarrison, R., 'Ind. Jour. Med. Res.,' vol. 7 (3), p. 633 (1920).

type has received confirmation from the work of E. and M. Mellanby, who have recently* reported identical thyroid changes in puppies to whose food butter had been added. Mellanby has further recorded that not only does butter, and in a lesser degree certain other fats, bring about these changes but they do not arise when the fat used is cod-liver oil.

The innocuous action of cod-liver oil may possibly have some relation to the iodine-content of this oil, which in crude specimens may reach a concentration of 1 in 2,000. It was, therefore, thought desirable to examine, by experimental methods, what the relationship between fat metabolism and iodine intake might be, and the relationship of thyroidal activity to both.

The present communication is a preliminary account of the observations so far made. It is yet too early to provide an explanation of them, but their record will serve to direct attention to etiological and nutritional problems concerned with what may be called the "fat-iodine-thyroid balance."

Tadpoles were selected as suitable for the purposes in view by reason of the fact that their rate of growth and of metamorphosis is so sensitive to influence by iodine and thyroid extract. It was thought that this sensitiveness might serve as an index of the changes that are induced in the thyroid gland by an excess of fats.

Details of Experiments.

(1) Butter, lard, cocoa-nut oil, oleic acid, linseed oil, cod-liver oil, and arachis oil were chosen as the fats to be tested; these have a wide range of iodine values. The cod-liver oil was of the crude variety—the so-called "cattle cod-liver oil"—its iodine-content was estimated by Mr. A. A. F. Peel and found to be 0·002 per cent. He also found that the linseed oil contained no iodine.

(2) The tadpoles were hatched from the same batch of eggs. They were selected so as to be of approximately the same size. The experiments commenced on the eleventh day of their life.

(3) Fifty tadpoles were placed in each of the twenty-four dishes required for the experiments. There was one litre of tap-water in each dish. The dishes were arranged in three series of eight each. The water was changed daily, and the dishes thoroughly cleansed.

(4) A basal diet was provided consisting of flour, eighty-five parts, and caseinogen, fifteen parts. Fresh pond weed was supplied: during the first six weeks it was changed every third day, thereafter daily. This diet contained an adequate supply of proximate principles, of vitamins and of vegetable matter. The tadpoles ate it with avidity and their rate of growth was vigorous. They were fed daily.

* Mellanby, E. and M., 'Proc. Physiol. Soc.,' vols. 7 and 10 (March 12, 1921).

(5) The fats to be tested were added (either alone or after admixture with known quantities of iodine) to the basal diet in the proportion of approximately 1 grm. of the fat to 1 grm. of the flour-caseinogen mixture. A dough of soft consistency was prepared therefrom, and the mass divided into a number of small pills which were supplied to the tadpoles.

(6) In the first series of eight dishes the effects of the various fats, as compared with controls receiving the same basal diet but without fats, were observed (fig. 1).

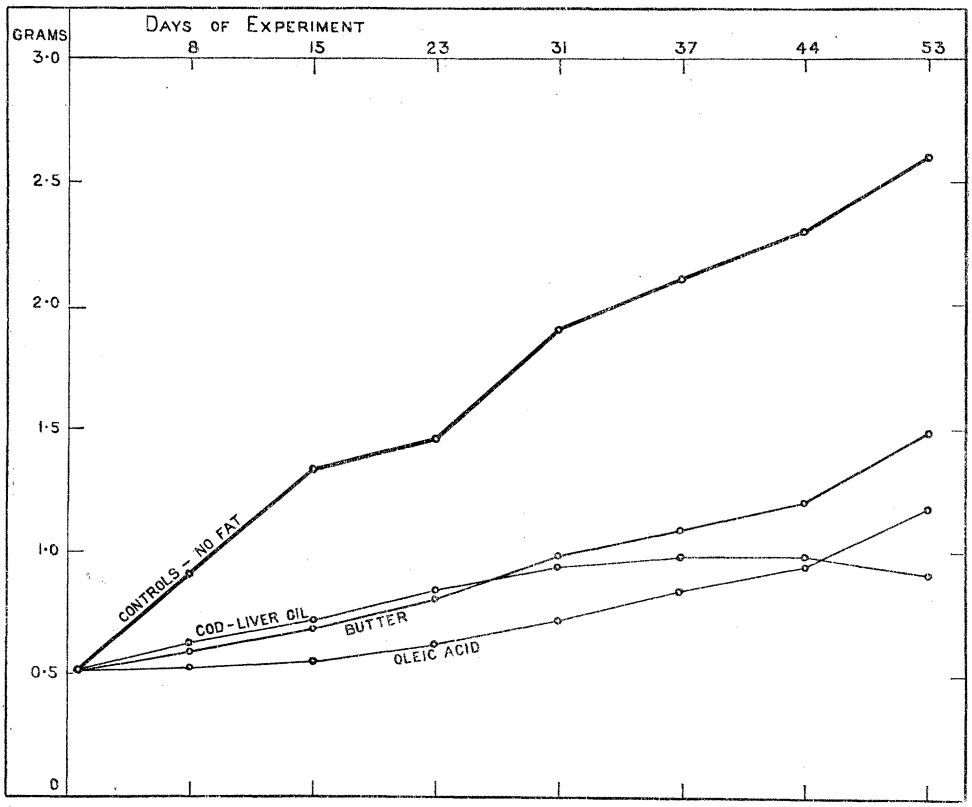

FIG. 1.—Showing the effects on growth of tadpoles of an excess of *oleic acid*, of *butter*, and of *cod-liver oil* in the food.

(7) In the second series of eight dishes the effects of the various fats after admixture with iodine, in the proportion of 0·5 mgrm. per gramme of food-mixture, were observed in contrast with controls receiving the same amount of iodine in the basal diet but without fats (figs. 2, 3, 4).

(8) In the third series of eight dishes the effects of the various fats after admixture with iodine, in the proportion of 1 mgrm. per gramme of food-

mixture, were observed in contrast with controls receiving the same amount of iodine in the basal diet but without fats (figs. 2, 3, 4).

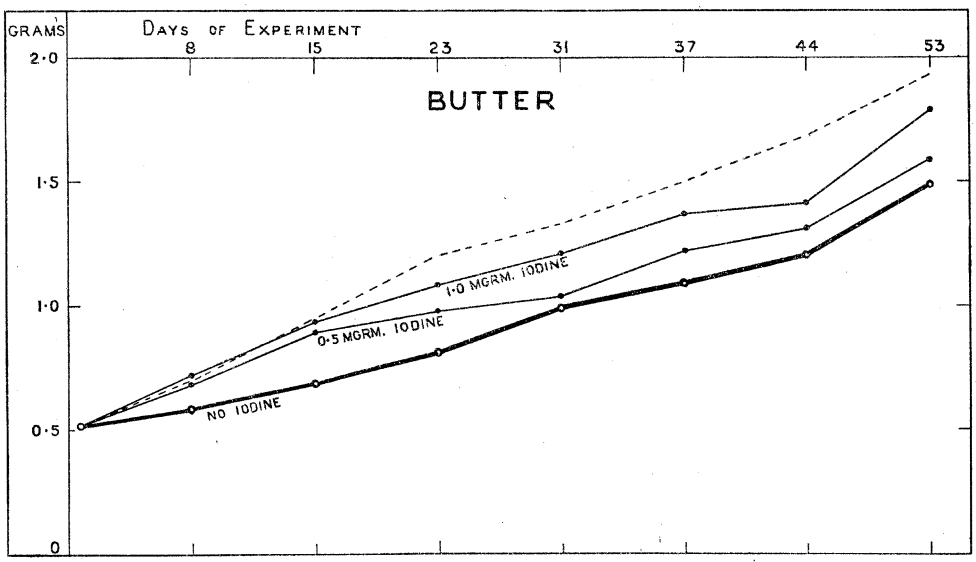

FIG. 2.—Showing the effects of 0·5 mgrm., and of 1·0 mgrm. of iodine in counteracting the retardation of growth induced in tadpoles by an excess of *butter* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but *without butter*.

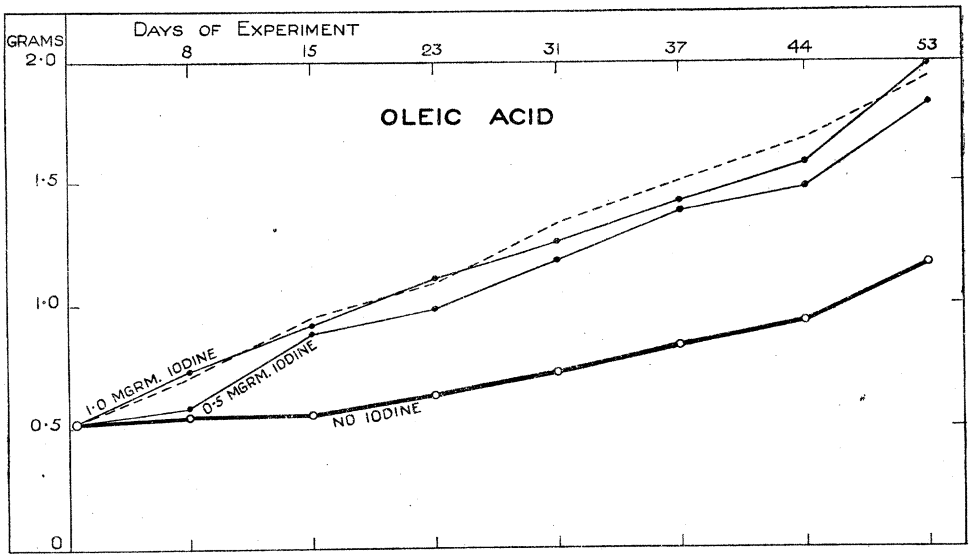

FIG. 3.—Showing effects of 0·5 mgrm. and of 1 mgrm. of iodine in counteracting the retardation of growth induced in tadpoles by an excess of *oleic acid* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but *without oleic acid*.

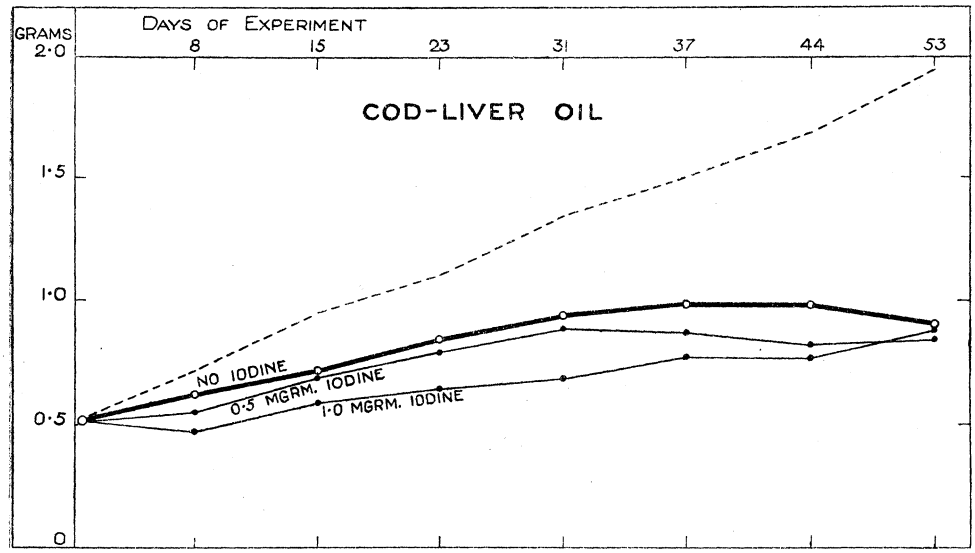

FIG. 4.—Showing the effects of 0·5 mgrm. and of 1 mgrm. of iodine in accentuating the retardation of growth induced in tadpoles by an excess of *cod-liver oil* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but without *cod-liver oil*.

(9) The iodine was added in solution of which the following is the composition: iodine, 0·477 grm.; potassium iodide, 0·682 grm. (equivalent to 0·521 of iodine); water, 100 c.c. The iodine solution was intimately mixed with the fat in each case, and the flour-caseinogen mixture subsequently added to form the soft pills.

(10) Food intake: The tadpoles ate the food-mixtures with avidity during the earlier part of the experiment, and during this period the rates of growth varied in a conspicuous way (fig. 1). Later, however, certain differences in the food intake in different dishes were observed. Tadpoles receiving the basal diet without admixture with fats, ate well throughout the whole course of the experiment, so also did those receiving food-mixtures containing arachis oil. Those receiving cod-liver oil and linseed oil ate greedily at first and later much more sparingly; while those receiving the harder oils, butter, cocoa-nut oil and lard ate moderately, as did those receiving oleic acid mixtures. It is concluded, therefore, that while the wide variations in the rate of growth (fig. 1) between tadpoles receiving no fat and those receiving fat may, in some measure, have been due to a lesser food intake by the latter during the later stages of the experiment, the great retardation of growth induced by the fat was not due in the main to this cause.

(11) Temperature conditions—which have been shown by Julian Huxley

to modify greatly the rate of growth of tadpoles—were, as far as possible, uniform. In the case of the lard and arachis oil dishes, the animals had the advantage of more heat from the sun, as these dishes were placed on a bench near a sunny window. In general, however, the factor of variations in external temperature was not responsible to any appreciable degree for variations in the rates of growth or metamorphosis. The room temperature during the course of the experiments ranged between 65° and 70° F.

(12) The mortality was negligible during the first three weeks of the experiment; later, and especially after the fortieth day, it was considerable amongst tadpoles receiving fluid fats, much less so amongst those receiving the solid fats. The presence of iodine in the food-mixtures tended to reduce the death rate in tadpoles receiving fluid fats. Œdema was a frequent cause of death in certain dishes: controls, cod-liver oil, arachis oil and lard. It appeared to have no relation to the presence or absence of iodine. Cannibalism, so usual among tadpoles, was conspicuous by its almost total absence, a circumstance which demonstrates the complete nature of the food provided.

(13) Pigmentation varied greatly in different dishes: the most pigmented were in general the smaller individuals and those receiving the fluid fats; the least pigmented were in general the larger individuals and those receiving the solid fats. Tadpoles receiving butter were, on the whole, less pigmented than those receiving other fats, although exceptions to this generalisation occurred among tadpoles receiving food-mixtures containing the more solid fats. It seems not improbable that the various fats may have had different effects on the pineal body.

(14) Individual variation in size was a very notable feature in the case of tadpoles receiving oleic acid, whether with or without iodine. It was a noticeable feature also in controls receiving no fats, and in those receiving food-mixtures containing lard, cocoa-nut oil and butter. Little or no variation in size was found among tadpoles receiving food-mixtures containing arachis oil, linseed oil and cod-liver oil.

(15) The method of recording rates of growth was as follows: The tadpoles were weighed weekly. At the first two weekly weighings only twenty from each dish were weighed. Subsequently—and as variations in the rate of growth of different individuals became more obvious—all were weighed. They were removed from their dishes into a gauze net. The wet mass of tadpoles at the bottom of the net was then freed of adherent water by means of absorbent paper, and the mass turned into a known weight of water. The total weight was thus arrived at, and the weight of twenty was calculated therefrom for purposes of charting. This figure was selected so as

to avoid errors such as might arise in consequence of reduction in the original numbers by a high mortality in any particular dish.

(16) Finally, an experiment was devised to determine the effect of fats in hastening or retarding the abnormally rapid rate of metamorphosis induced by a large intake of iodine. In this experiment twenty tadpoles were used for each fat to be tested. The diet consisted of a modification of that originally used by Swingle, who produced metamorphosis very rapidly by means of a mixture of one part of metallic iodine to 100 parts of flour. In the present experiment the same proportion of iodine was employed, but with this difference, that instead of flour, a mixture of flour and caseinogen, in the proportion of eighty-five parts of the former to fifteen parts of the latter, was used. The fats to be tested were added in quantities sufficient to make a soft dough. Pond weed was not given to any of the tadpoles in this series. The fats tested were butter, cocoa-nut oil, oleic acid, linseed oil and cod-liver oil. The observations were controlled by forty tadpoles, twenty of which received the basal diet without fat or iodine, and the remaining twenty the basal diet with iodine but without fat.

Results of the Experiments.

A. *Observations on Growth:—*

(1) The rate of growth of tadpoles receiving an excess of the fats, in an otherwise adequate dietary, was greatly delayed (fig. 1). This retardation of growth was not attributable solely to a lesser food intake, although this may have been responsible in some measure for it, but was dependent on other and unknown causes. The fats tested ranged themselves in the following order with respect to the severity of the retardation of growth induced by them during a period of fifty-three days: arachis oil, cod-liver oil, linseed oil, cocoa-nut oil, oleic acid, lard and butter.

(2) Iodine in the proportion of 0·5 to 1·0 mgrm. per gramme of food-mixture compensated, in greater or lesser degree, for the retardation of growth induced by certain fats, namely, butter, oleic acid, cocoa-nut oil, lard and arachis oil (figs. 2 and 3). On the other hand, no favourable influence on the rate of growth was exercised by this dosage of iodine in the presence of linseed oil and cod-liver oil; the rate of growth was, on the contrary, further retarded (fig. 4). The effect of iodine in compensating for the retarded growth induced by fats was due either to its action in increasing the intake of food or to its action in improving food-assimilation, or to both. With respect to this action of iodine, the fats used group themselves into two categories: those in whose presence food-assimilation was favoured by

iodine; and those in whose presence food-assimilation was not favoured by iodine. The first category is further divisible into two sub-classes, according to the degree of the favouring influence of iodine on assimilation: those in which the retarded rate of growth induced by the fat was wholly or almost wholly compensated for by 1·0 mgrm. of iodine per gramme of food-mixture; and those in which the retarded rate of growth was favourably influenced, but not compensated for, by this dosage of iodine. In the first sub-class are included butter, lard and oleic acid (figs. 2 and 3); in the second, cocoa-nut oil and arachis oil.

B. *Observations on Metamorphosis.*

(1) Among tadpoles which received metallic iodine in the proportion of one part of iodine to 100 parts of food-mixture, but no pond weed, the following results were observed at the end of thirty days of experiment:—

(*a*) Tadpoles whose food-mixture contained butter showed well-developed hind limbs in every case; the stage of metamorphosis was in advance of controls which received the same amount of iodine but no fats, and was far in advance of those which received other fats. Butter thus hastened the abnormal metamorphosis induced by a large iodine intake.

(*b*) A similar but less uniform result was observed with regard to cocoa-nut oil. This oil hastened in certain individuals the normal metamorphosis induced by a large iodine intake.

(*c*) Oleic acid and linseed oil exercised little or no influence on the abnormally rapid metamorphosis induced by a high iodine intake.

(*d*) Cod-liver oil, on the other hand, markedly delayed the abnormally rapid metamorphosis induced by a high iodine intake, and at the same time the loss of weight induced by the iodine was less pronounced than in the case of other fats, oleic acid excepted.

It was observed that the hind limbs developing in the course of this experiment were unhealthy looking and sometimes shrivelled, especially in the case of linseed oil.

(2) Among tadpoles which received 0·5 to 1 mgrm. of iodine per gram of food-mixture and pond weed, the rate of metamorphosis was little affected in the absence of fats; the larger dose of iodine tended to hasten metamorphosis slightly. When, however, fats were present, it was observed that metamorphosis was markedly delayed by the fluid fats, and to a much less extent by the harder fats; and, that while iodine tended to compensate for the delay in metamorphosis induced by oleic acid and arachis oil, no such

tendency to compensation occurred in the presence of linseed oil and cod-liver oil.

Summary of Results.

(1) An excess in the food of tadpoles of the several fats used caused great retardation in the rate of growth.

(2) Iodine in amounts of 0·5 to 1·0 mgrm. of food-mixture tended to compensate for the retardation of growth induced by butter, lard, oleic acid, cocoa-nut oil, and arachis oil, but not for that induced by linseed oil and cod-liver oil.

(3) The normal rate of metamorphosis was but slightly affected by the harder fats: butter, cocoa-nut oil, and lard; but was delayed by the fluid and less saturated fats: oleic acid, arachis oil, linseed oil, and cod-liver oil.

(4) The delayed rate of normal metamorphosis induced by the fluid fats tended to be compensated for by the presence of small quantities of iodine in the food in the case of oleic acid and arachis oil, but was not compensated for by the same quantities of iodine in the case of linseed oil and cod-liver oil.

(5) The abnormal metamorphosis induced by a high iodine intake was considerably hastened by a high proportion of butter in the food-mixture, and to a lesser degree by a high proportion of cocoa-nut oil in the food-mixture, but was markedly retarded by a similar proportion of cod-liver oil in the food-mixture.

Conclusion.—It seems probable from these results that, in so far as certain fats—butter, lard, oleic acid, cocoa-nut oil, and arachis oil—are concerned, an iodine intake, proportionate to their intake in the food, is requisite for the maintenance of normal metabolism. The influence of cod-liver oil and of linseed oil in further retarding growth in the presence of an amount of iodine, that is favourable to growth in the case of other fats, is as yet not understood.

The histological findings in connection with the thyroid gland will form the subject of a further communication.