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The Effect of Rapeseed Oil on the Thyroid Function of Rats

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With 2 figures and 1 Table

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It has long been recognized that the seeds of *Brassica* spp. cause enlargement of the thyroid gland, the condition being referred to as "Brassica goitre". Nowadays, it is known that the seeds of many of these species contain some compounds which inhibit the function of the thyroid. One of these goitrogens has been identified as L-5-vinyl-2-thiooxazolidone (Astwood et al. 1949). It is interesting to note that this antithyroid agent is a sulphur compound (thionamide), as was originally suspected, although it was at first thought to be one of the mustard oils. It is not yet known with certainty whether this thiooxazolidone is the chief or possibly the only goitrogen in the *Brassicae*.

Thiooxazolidone is also present in the seed of turnip rape, which is a species of *Brassica*. The goitrogenic activity of rapeseed had been noted in a number of earlier studies and it is generally agreed that it produces its effect by interfering with the synthesis of thyroxine (Greer 1950). Added iodine does not seem to prevent rapeseed from inducing enlargement of the thyroid. A particularly strong effect has been observed on feeding rapeseed meal. When the effect on chicks of the rapeseed oil extracted from the whole seed meal was compared with that of the extracted meal, the activity of the latter was found to be 2–3 times greater (Turner 1948). It is considered likely that the sulphur compounds in rapeseed either disappear during the extraction and refining of the oil or remain in the meal (Lips 1952).

The physiological effect on rats of rapeseed oil itself has attracted the interest of many workers. Rapeseed oil has been shown to decrease the rate of growth of young rats, to be poorly digested and absorbed, and to increase the cholesterol content of the adrenal glands of the animals (Beznak et al., 1943; Carrol, 1951; Deuel et al., 1948; Thomasson, 1955, Roine and Uksila, 1959; Deuel et al., 1940; Thomasson, 1956). Carrol and Noble (1947) reported that the main component of rapeseed oil, erucic acid, sometimes caused a relative increase in the weight of the thyroid. The discovery raised the question of whether rapeseed oil might possibly interfere with thyroid function, and of whether its effect on the thyroid might be connected with its other known physiological effects on rats (cf. above). The object of the present study was to find an answer to these questions by making use of the radio-iodine (^{131}I) technique.

Experimental

Experimental Animals. The animals used in the experiments were young male rats of the Sprague-Dawley strain. In the different experiments the initial weight of the animals varied from 50—70 gm., but in no single experimental group did differences in weight exceed 10 gm.

Diet. The following basic diet was used in the experiments: 580 gm. of graham flour, 100 gm. of dried brewer's yeast, 150 gm. of casein and 20 gm. of a salt mixture (100 gm. of sodium chloride, 100 gm. of calcium lactate, 30 gm. of ferric citrate, 10 gm. of manganese sulphate, 2 gm. of copper sulphate, 0.2 gm. of potassium iodide). To 850 gm. of this basic diet were added 306 gm. of rapeseed or soybean oil and 2 gm. of cod liver oil to supply vitamins A and D. Thus the added fat contributed 50 cal.-per cent to the diet.

Tap water was given *ad libitum*.

In some experiments the oil was replaced by a mixture of either a) saponified rapeseed oil fatty acids (91.3%) plus 8.7% glycerol or b) the unsaponifiable fraction of rapeseed oil (0.8%) plus soybean oil (99.2%). In these experiments, also, the added fat amounted to 50 cal.-per cent of the diet.

The unsaponifiable fraction was separated from the rapeseed oil by first saponifying in the usual way, i. e. boiling for 3 hours in a ca. 0.5 N alcoholic solution of potassium hyroxide, and then distilling off about half the ethyl alcohol. Whilst the soap solution was still warm, it was diluted with a large amount of warm water. The unsaponifiable fraction was recovered by the A. O. A. C. method (1950), the warm diluted soap solution being extracted three times with peroxide-free ethyl ether. The combined ether extracts were washed, first 3 times with distilled water, then 3 times with 0.5 N aqueous potassium hydroxide solution, and finally with distilled water until the washing water no longer gave a basic reaction with phenolphtalein. The extract was dried with sodium sulphate and the ether distilled off on a water bath (40—50°C) at slightly reduced pressure, the residue being dried at 80°C. From the rapeseed oil used 0.8 per cent of unsaponifiable substances was recovered.

When the unsaponifiable fraction had been separated, the fatty acids were liberated with 10% hydrochloric acid. The solution was allowed to stand at a temperature of ca. 5°C, and the fatty acids rising to the surface were removed, washed and dried. To avoid loss, the acid water layer and washing waters were extracted with peroxide-free ethyl ether.

Administration and measurements of radio-iodine. Radio-iodine, ^{131}I, was injected intraperitoneally into lightly anaesthetized test animals. The dose was from 5.5—6.5 μc per animal, and the volume of the injection 1.0—1.4 ml. The radio-iodine used was carrier-free and dissolved in thiosulphate solution (pH 8—10), diluted just before injection with physiological saline. The radioactivity of the thyroid gland was measured on unanaesthetized animals in the neck region at the point where the counts were found to be maximal. The measurements were made with a scintillation counter in which there was a sodium iodide (Tl) crystal. The counter was enclosed in a lead shield 6 mm. thick, covered with a layer of brass 2 mm. thick, in the base of which there was a round opening 16 mm. in diameter.

The radioactivity of the thyroid gland was measured by determining the time required for a total count of 10^4 impulses. From this the cpm value was calculated and corrected for background and decay. For each animal 2—4 replications of every measurement were made, the cpm value being calculated from the highest of these. The replicate measurements were usually very close, any slight variations probably being due to the restlessness of the animals. All the cpm values were at least 10 times higher than the background values.

The radioactivity was measured 3, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours after the injection of radio-iodine. This made it possible to follow both the uptake of the radioiodine by the thyroid and its disappearance. The value for 3 hours, corrected for background and decay, was taken as 100 per cent and the values at 4, 6, 12, 24 and 48 hours, duly corrected, were expressed as percentages relative to this. In this way, correc-

tions for differences in weight and dosage of radio-iodine could be avoided. The percentages obtained were plotted as a function of time on arithmetic scales, to give the radio-iodine uptake curve. If the corrected values are plotted against time on a semi-logarithmic scale, the curve is almost linear after the peak is reached, and from it can be estimated the biological half-life of ^{131}I, i. e. the time in which the radioactivity of the thyroid gland is halved. In this case, the value at 48 hrs proved to be the best for purposes of comparison. This value, corrected for background and decay, was taken as 100 per cent, and the subsequent corrected values calculated relative to it. The percentages obtained then revealed how much radio-iodine still remained in the gland on successive days compared with that present 48 hours after the injection.

Results

A. Soybean oil and rapeseed oil

After some 23 days (limits 18–28 days) on the experimental diet containing 50 cal.-per cent of soybean oil or rapeseed oil, the animals were weighed and transferred to individual cages whilst the radio-iodine experiments were being performed. The average weight of the 24 animals of the soybean oil group was then 134.4 gm. and that of the 21 rats of the rapeseed oil group 107.0 gm. The rate of growth in the rapeseed oil group in these experiments was thus, as usual, considerably slower than in the soybean oil group.

Uptake of radio-iodine by the thyroid gland. The curves showing the uptake of radio-iodine, obtained in the manner described above, are represented in Figure 1 in the form of mean curves. There are clear-cut differences between the two groups. In the rats that had received soybean oil there was a continuous rise in the uptake of ^{131}I to a peak value about 24 hrs after the injection (of the 24 rats of this group, only one had a maximum value at 12 hours and one at

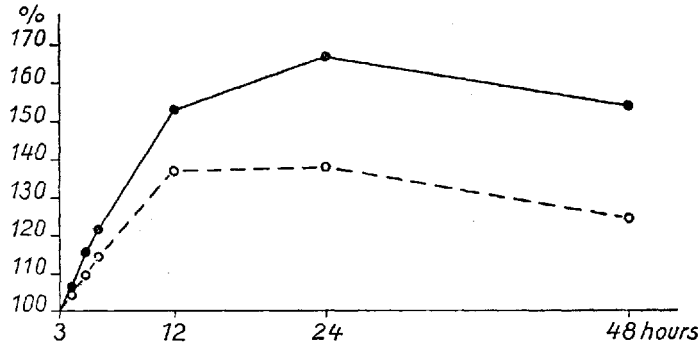

Fig. 1. Uptake of ^{131}I in rats fed on soybean oil (solid line) or rapeseed oil (broken line).

48 hours). In the rats fed on rapeseed oil the peak value was reached considerably earlier, presumably at about 12 hours, after which the radioactivity values seem to remain approximately constant for the next 12 hours.

From the uptake curves it can further be seen that the soybean oil group accumulated relatively more iodine than the rapeseed oil group (soybean curve maximum about 167%, rapeseed curve about 138%) and at a greater rate (slope of the curve between 6–12 hours ca. 1.6 in the soybean oil group, and ca. 1.1 in the rapeseed oil group). Because the values for the iodine uptake

in these curves were obtained by comparison with the values 3 hours after injection and are hence only relative, it should be noted at this point that the absolute values for the rapeseed oil group were also smaller than for the soybean oil group.

The disappearance of radio-iodine from the thyroid. The mean value for the biological half-life of ^{131}I (the radioactivity of the thyroid 48 hrs after the injection being taken as a standard of comparison) was in the soybean oil group 5.4 $\pm$ 0.2[1]) days and in the rapeseed oil group 4.6 $\pm$ 0.2 days. The difference was statistically significant ($P < 0.02$).

If, moreover, the rates of diminution of thyroidal ^{131}I were determined and expressed in terms of percentage per day, it was found (Table 1) that on the first day, i. e. between 48 and 72 hours after injection, the radioactivity

Table I

Rates of diminution of thyroidal I^{131} in terms of percentage per day.

	Hours				
	48—72	72—96	96—120	120—144	144—168
Soybean oil	13.3	11,2	8.2	7.8	6.7
Rapeseed oil	16.1	12.0	9.6	6.7	6.2

of the glands of the rats fed on rapeseed oil had diminished considerably more (16.1%) than that of the rats receiving soybean oil (13.3%) ($P < 0.05$). But after this point, there was no appreciable difference in the rate of diminution, except possibly on the fourth day (120—144 hours, $P < 0.1$).

In order to gain some insight into the mode of action of rapeseed oil, an experiment was carried out in which one group of animals was kept on the rapeseed oil diet and a control group was given a soybean oil diet to which was added 0.1% or 0.05% of propylthiouracil. Propylthiouracil (PTU) is a substance known to inhibit the synthesis of thyroxine effectively without interfering with the ability of the thyroid to accumalate inorganic iodine (VANDERLAAN and VANDERLAAN, 1947; TAUROG et al. 1947; TAUROG et al. 1951).

Fifteen hours after the injection of radio-iodine, the rats were given an injection of 10 mg. of potassium thiocyanate, which is known not only to inhibit the process of iodine accumulation but also to cause the discharge from the thyroid gland of the inorganic iodine already contained in it (WOLFF et al. 1946; VANDERLAAN and VANDERLAAN, 1947). Under the influence of KSCN, the radioactivity of the thyroid gland fell during the first hour by an average of 70 per cent (with 0.1% PTU) or 57 per cent (with 0.05% PTU) in the soybean oil group, but only by 3.6 per cent in the rapeseed oil group. These figures show that 15 hours after the injection over half the ^{131}I was still in inorganic form in the soybean oil group. In the rapeseed oil group, by contrast, the proportion was only 3.6 per cent, the bulk of the ^{131}I (over 96 per cent) having been bound in organic form. It is clear, therefore, that the mode of action of

[1]) Standard deviation of the mean.

rapeseed oil is different from that of PTU; it does not prevent the synthesis of thyroxine, but interferes with the ability of the thyroid gland to accumulate iodine.

B. The saponifiable and unsaponifiable fractions of rapeseed oil

Experiments were designed to indicate which of the components of rapeseed oil is mainly responsible for this difference between the soybean oil and rapeseed oil group. In one group of animals (A), the rapeseed oil in the diet was replaced by the unsaponifiable fraction derived from it (0.8%) plus soybean oil (99.2%), and in another group (B) by the fatty acids obtainded from the saponifiable fraction (91.3%) plus glycerol (8.7%).

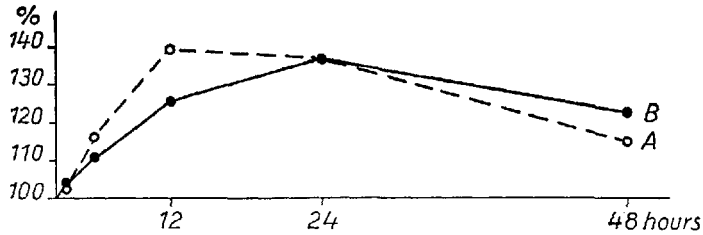

Fig. 2 Uptake of ^{131}I in rats fed on unsaponifiable fraction of rapeseed oil plus soybean oil (A), or saponified rapeseed oil fatty adcis plus glycerol (B).

About 20 days later (limits 17—24 days) the animals of group A were tested with radio-iodine, and after 27 days (limits 24—28 days) the animals of group B were similarly tested. The average weight of the animals was then 140.3 gm. in group A (7 rats) and 80.3 gm. in group B (8 rats). The saponifiable fraction thus appeared to reduce the rate of growth of the rats appreciably.

Uptake of radio-iodine by the thyroid gland. For the animals of group A, which had received soybean oil plus the unsaponifiable fraction of rapeseed oil, the curve for the uptake of radio-iodine (Figure 2) was exactly similar to that for the rapeseed oil group. The peak was about 139 per cent (with rapeseed oil 138 per cent), the radioactivity remained roughly constant between 12 and 24 hours, and the slope showing the rate of uptake (6—12 hrs) was about 1.2 (for the rapeseed oil group 1.1).

By contrast, in group B, in which rapeseed oil had been replaced by the fatty acids of the saponifiable fraction plus glycerol, the uptake curve resembled that for the soybean oil group, except that the iodine uptake between 6 and 12 hours was slower and throughout relatively smaller.

Disappearance of radio-iodine from the thyroid gland. The biological half-life of ^{131}I in Group A was 4.4 $\pm$ 0.3[1]) days and in Group B 6.0 $\pm$ 0.7 days. The unsaponifiable fraction of the rapeseed oil (Group A) had thus reduced the half-life in the soybean group to the same level as that of the rapeseed oil group, i. e. from 5.4 days to 4.4 days (the values being relative to the radioactivity at 48 hrs). The difference between these values is statistically significant ($P < 0.05$). In Group B (saponifiable fraction of rapeseed oil plus glycerol) the half-life was somewhat greater even than the value in the soybean oil group itself (5.4 days), although this difference is not statistically significant.

[1]) Standard deviation of the mean.

Discussion

The results show that rapeseed oil, and specifically its unsaponifiable fraction, interferes with the thyroid gland function when fed to young rats, the uptake of ^{131}I being smaller and slower than in a control group of rats receiving soybean oil. In addition, radio-iodine disappears from the thyroid gland more rapidly in rats on a rapeseed oil diet.

In the light of the experiments, the mode of action of rapeseed oil seems probably to consist in partial inhibition of the ability of the thyroid to absorb iodine from the plasma. For this reason the iodine supply in the gland is insufficient, and the output of thyroxine below normal. This leads to an increase in the secretion of thyrotrophic hormone (TSH) by the hypophysis and in the concentration of this hormone in the blood. TSH is known to exert at least two effects: it increases the ability of the thyroid gland to accumulate iodine by inducing hyperplasia of its epithelium and it stimulates the release of thyroxine into the blood. In the rats on the rapeseed oil diet thyroxine is released from the glands faster than from those of the controls, as is attested by the shorter biological half-life of ^{131}I, 4.6 days as compared with 5.4 days in the controls. Since these values were calculated relative to the radioactivity at 48 hours, it can be taken as certain that the whole radioactivity represents organically bound radio-iodine or ^{131}I-labelled thyroxine (Wolff, 1951; Taurog and Chaikoff, 1947, 1948; Taurog et al. 1950) and that the rate of decline may be taken as an index of hormone secretion by the gland.

The actual synthesis of thyroxine or the binding of ^{131}I in organic combination is apparently not inhibited. This view is supported by the results of the experiments with potassium thiocyanate, which showed that only 3.6 per cent of the radio-iodine accumulated in the glands of the rats on a rapeseed oil diet was still in inorganic form 15 hours after the injection. In the control group on soybean oil, in which the capacity of the thyroid to synthesize thyroxine was partially inhibited with propyl-thiouracil, more than half the radio-iodine was in inorganic form. There are thus no grounds for alleging inhibition of thyroxine synthesis as the cause of the partial deficiency of thyroxine in the rats fed on rapeseed oil. This deficiency is attributable to the lack of iodine caused by the partial inhibition of the ability of the thyroid gland to accumulate iodine. Evidence that the available thyroxine is below the normal level in rats fed on rapeseed oil is also possibly afforded by the observation of Carroll and Noble (1952), that 1 per cent of dried thyroid was toxic to the control group, but not to the animals on a rapeseed oil diet.

The influence of rapeseed oil on the activity of the thyroid gland cannot be regarded as especially great, but in the light of the experiments here described it clearly seems to lead to a state of hypothyroidism. To what extent this phenomenon is responsible for the other physiological effects of rapeseed oil cannot be decided from the present results.

Summary

When young male rats were fed on a diet containing 50 cal.-per cent of soybean oil or rapeseed oil, carrier-free radio-iodine, ^{131}I, injected intraperitoneally in doses of 5.5—6.5 μc per animal, accumulated in the thyroid glands of the rapeseed oil group at a slower rate and in smaller amounts than in the control group fed on soybean oil. The radioactivity of the glands of the rapeseed oil group remained approximately constant between

12 and 24 hours after the injection, whereas the activity of the glands of the control group continued to increase up to a maximum at about 24 hours.

The rate of depletion of the thyroidal radio-iodine was greater in the rapeseed oil group than in the soybean oil group. The biological half-life of ^{131}I was found to be 4.6 days in the rapeseed oil group and 5.4 days in the soybean oil group, when calculated relative to the radioactivity of the thyroid 48 hours after the injection.

When the rapeseed oil diet was replaced by the corresponding saponifiable fraction plus glycerol or by adding the unsaponifiable fraction of rapeseed oil to soybean oil, the biological half-life of ^{131}I was found to be 6.0 dyas in the former and 4.4 days in the latter. Thus the unsaponifiable fraction of rapeseed oil reduced the half-life in animals fed on soybean oil to the same level as that in animals fed on rapeseed oil. The effect of this same fraction on the iodine uptake curve of the soybean oil group was also to modify it so that it resembled the uptake curve of the rapeseed oil group.

The results obtained indicate that rapeseed oil, and specifically its unsaponifiable fraction, contain a factor (or factors) which interferes with the ability of the thyroid gland to accumulate iodine from the plasma, but which does not seem to prevent the binding of iodine in organic form. For this reason, the thyroid gland itself becomes deficient in iodine, with the consequence that the synthesis of thyroxine is reduced and the secretion of thyrotrophic hormone increased. The result is hypothyroidism, despite the presence of an adequate amount of iodine in the diet.

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