# SHORT COMMUNICATIONS

## A NEW METHOD FOR REFINING OF SHARK LIVER OIL ON COMMERCIAL SCALE AND RECOVERY OF VITAMIN 'A' BY PARTIAL SAPONIFICATION

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### Introduction

Despite synthetic preparation, fish liver oil will continue to be a potential source of vitamin A in the world market. The shark liver oil industry in Pakistan has a great future. The methods for extraction and refining of shark liver oil as practised in other countries do not however seem economical in this country. Sharks from tropical waters yield liver oil comparatively of higher melting point. Only a small fraction of oil can be obtained in liquid state when wintered at 20-22°C. The major portion of crude oil remains solid, and unless vitamin A could be recovered from solid fraction easily, the liver oil industry will not be a sound proposition.

The method described in the present communication is inexpensive and easy to operate without much installation. It works on the principle of extraction of vitamins A and D from partially saponified crude oil through the medium of cottonseed or any liquid vegetable oil. By extracting three times in succession, a transference of vitamin A to the extent of 95% has been achieved. For partial saponification, caustic potash or caustic soda may be used, although the former works better. A patent has been applied for in Pakistan under Patent Application No. 1082/59, dated 3.12.1959.

### Experimental

In a 15-literdrum, provided with two taps, were placed 5 lbs. of crude shark liver oil (4,000 I.U./g.). 145 g. of caustic soda dissolved in 150 ml. of water was introduced and stirred thoroughly. The mixture was allowed to stand for 48 hours. Five lbs. of cottonseed oil heated to 60°C. was added and the whole made into a uniform mass by stirring. This was allowed to stand for 4 hours, and 2 liters of hot saturated brine was then added to precipitate the soap into a granular mass. After standing for 24 hours, 5 lbs. of the supernatant liquid oil was removed through the upper tap and was collected in a second drum with two taps as before. The oil, washed first with hot brine (90°C.) and then with hot water, was separated by the upper tap and centrifuged in an oil centrifuge. This was termed as first extract. The potency of the oil was determined chemically using colorimetric comparison on the Lovibond tintometer, and found to be 2,500 I.U./g. Transference of vitamin was 62%. To the residual mass a further quantity of 5 lbs. of fresh cottonseed oil heated to 60°C. was added. The mass was stirred thoroughly and allowed to stand overnight. Five lbs. of oil was removed, washed and centrifuged as before. The potency of this second extract was determined and found to be 1,000 I.U./g. Transference of vitamin in this case was 25%.

The residual mass was treated for the third time with another lot of fresh cottonseed oil. The oil recovered was 5 lbs. with a potency of 300 I.U./g. Transference of vitamin in this case was 8%. The total transference of vitamin by these three partial treatments was 95%. The solidifying point of the crude oil was 30-35°C. and that of the product was 12°C.

By blending the oil impregnated with vitamin A obtained by the above treatments a standard product of average potency was produced. The third extract having a low vitamin potency was used for transference of vitamin in subsequent operations. To prevent any deposition of "solid oil" in cold weather, the oil was wintered at 16°C. The wintering period was 5 days at a stretch.

In the above experiment saponification had also been effected by caustic potash. The soap in this case separated from the unsaponified oil and was found as a separate layer at the bottom. No brine treatment was necessary. Separation of oil-solvent used for transference of vitamin proceeded with greater ease. Percentage of transference of vitamin A in the first extract was found to be slightly higher than that with caustic soda. The residue was utilised for the production of cheap quality washing soap as by-product.

The procedure was further utilised to produce

higher potency oil from lower one. Vitamin-bearing oil recovered as the first extract was used for transference of vitamin in the second operation. By extending the process to next operation a higher potency oil was produced.


## Summary

This is a process for refining and production of shark liver oil by partial saponification either with caustic soda or caustic potash. In this process cottonseed oil was used as an oil solvent for transference of vitamin from crude shark liver oil.

Higher vitamin A potency oil was also produced from lower potency crude shark liver oil by using this process.


## Reference


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# EUBLEMMA AMABILIS


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In a systematic cultivation of lac, the first point to remember is that it should be intensive. It means that there should be a forest-plan for the particular lac-plantation or lakhera, and naturally this cannot apply generally to each and every locality even when the insect and its host tree remain the same. The forest-plan will be an integration of numerous factors, some promoting the multiplication of the insect and others detrimental to its life, being a sort of balance sheet to indicate how favourable the enterprize is likely to be in the end.

Coming to the factors destructive to lac, a serious one is the attack from the predacious caterpillars of *Eublemma amabilis* moth. The destruction due to this enemy alone has to be quantitatively worked out and, before we can proceed with it, its population with each crop has to be calculated. No such attempt has been undertaken because of the difficulty of counting the caterpillars in a given specimen of fresh stick-lac where the lac insects are still alive. Normally the caterpillars of *E. amabilis* eat beneath the cover of the lac crust. This is apparently due to the moth larvae shunning light. When an isolated piece of fresh stick-lac is placed in a wooden box, dark and yet sufficiently airy, the caterpillars, instead of remaining within the lac, form perpendicular columns of tunnels projecting outside the twig.

In Mysore, the insect, *Lakshadia, mysorensis*, growing there on *Shorea talura*, suffers much from the attack of *E. amabilis*. As such, any given sample of fresh lac would contain sufficient predacious caterpillars of this moth. Figure 1 shows such a specimen with rod-like projections arising perpendicularly to the length of the twig. These

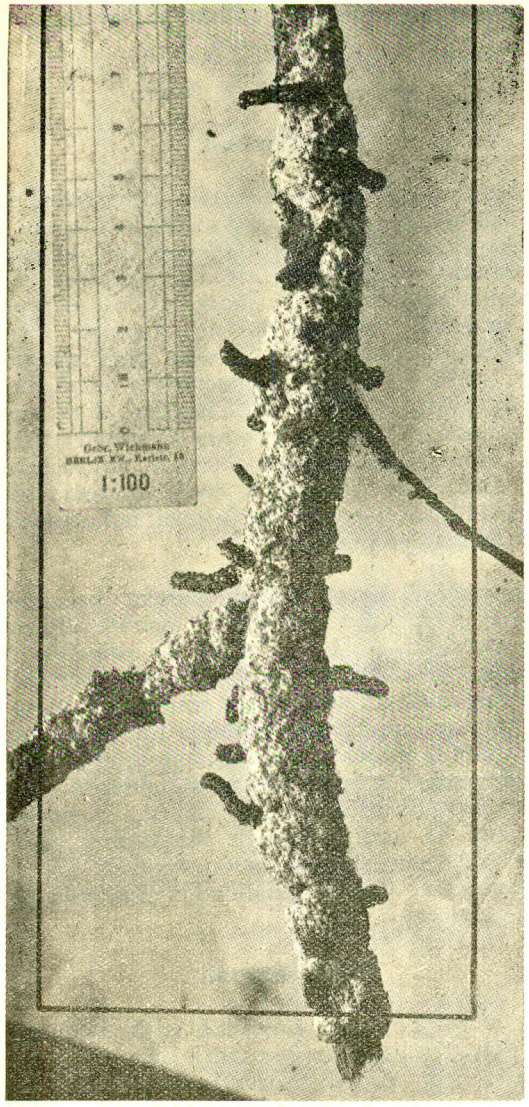



Fig. 1.—Fresh stick-lac of *Lakshadai mysorensis*, on *Shorea talura*, Bangalore. Sample of stick collected at the end of the rainy season. The worm-like projections are hollow tubes constructed by the caterpillar of *Eublemma amabilis*. They are made of resinous excreta of the caterpillar incorporated in a web-tube secreted by the insect. The scale shows centimetres.

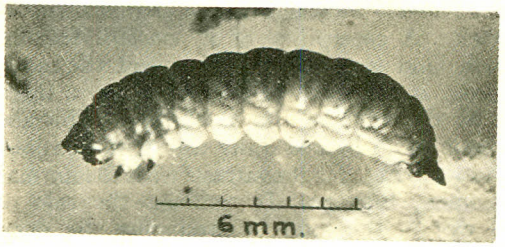
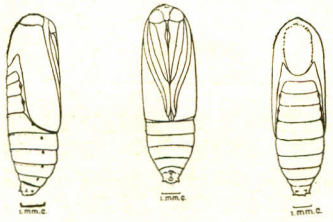



Fig. 2.—Caterpillar of *E. amabilis*, not full grown yet, with its anal end half ejecting a disc shaped excreta seen sideways.



(a) (b) (c)

Fig. 4.—The pupa of *E. amabilis* :— (a), seen sideways ; (b), from its ventral side; (c), from above.

projections are formed by the *E. amabilis* caterpillars, which secrete silken thread and bind their excreta together to construct hollow tube-like tunnels within which they move. Normally these tubes are hidden between the lac crust and the surface of the twig. When otherwise exposed, as in Fig. 1, the population of living caterpillars is at once ascertained, which is a very helpful device in quantitatively estimating the injury due to this enemy alone. I have not found any other enemy-caterpillar behaving likewise, certainly not that of *Holcocera pulverea*, which is also common in several localities. This caterpillar also forms a silken cacoon but purely of silk, and a very loose

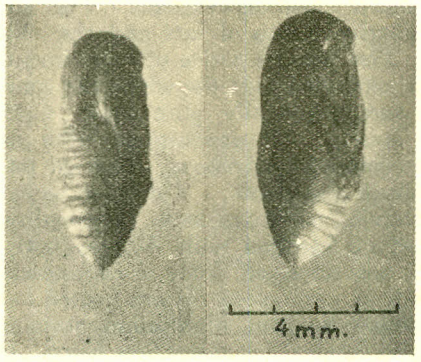



Fig. 6.—Pupae of *E. scitula*, that of male to the left, that of female to the right.

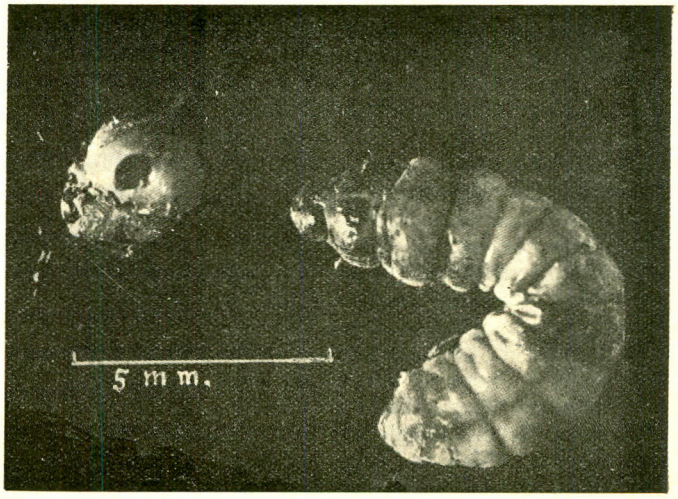
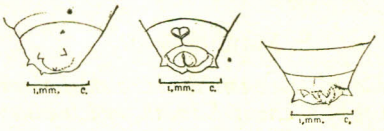



Fig. 3.—*E. amabilis* caterpillar, full grown, to the right. To the left a ball shaped object, the last moult skin of the caterpillar, with an oval dark object, the excreta.

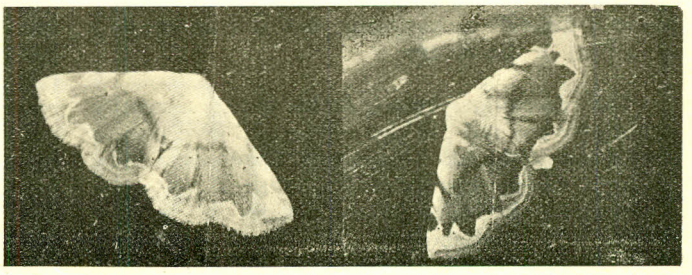


(a) (b) (c)

Fig. 5.—Pupa of *E. amabilis*, its posterior end enlarged. (a), seen sideways; (b), from beneath; (c), from above.

one. In the architecture of its cacoon, its excreta is not incorporated; this is of a granular nature hardly capable of offering any strength to a tube-like construction.

Figure 2 shows the caterpillar of *E. amabilis* not yet full grown. The anal end at right shows a disc half projecting out of the anus. It is the excreta of the caterpillar seen in profile. In



(a) (b)

Fig. 7.—Moths of *E. amabilis*, almost natural size. (a), the normal moth, deeply coloured; (b), from a caterpillar feeding on lac attacked by *Erencyrtus dewitzii*, the moth being yellow, instead of pink.

Fig. 3, at right is shown a full grown caterpillar, and at left a ball shaped object represents the moulted skin of the caterpillar before it became a pupa, with an oval dark spot, a single disc of excreta, which was seen sideways half ejected in Fig. 2. The malpigian tubes of the lac insect, contain the ether soluble portion of lac resin which is soft or semi-fluid but easily dries to a brittle surface when smeared on a microscopic slide. When the caterpillar of *E. amabilis* eats the lac insect it also feeds upon the contents of the malpigian tube which is the "soft resin" and it is this which is excreted as a hard disc. It may be noted that lac when burnt gives a pleasant butter-like smell, and on dry distillation of lac butyric acid has also been recovered. There is no doubt that the ether soluble constituent of lac is first to be found in the malpigian tube. When finally excreted it comes as flat discs, which are hard and severe, so to say, as bricks, which joined together by a silken web secreted by the caterpillar, goes to form a tube or tunnel, within which it moves while it is feeding more and more on the lac insects.

The same specimen, Fig. 1, for example, can be further dissected later on to see how many pupae of the moth were there, to supplement the finding based on caterpillars alone. Figure 4 gives a pupa of *E. amabilis* as seen in three poses: Fig. 4a, shows it sideways; Fig. 4b, ventrally; and Fig. 4c, dorsally. The anal end of the pupa is shown further enlarged in Fig. 5. Figure 5a gives the sideview; Fig. 5b, its ventral and Fig. 5c, its dorsal aspect.

From 1954 to 1959, I have reared parasites from both the annual crops and it has been my impression that *Eublemma amabilis* is least common in Sind. However, from no other lac could I breed *E. scitula* more than from Sind lac (pupae shown in Fig. 6. for comparison); it is fortunately rare even here. The caterpillars of *E. scitula* hardly feed underneath the lac encrustation. They usually devour young lac insects and are, so to say, surface feeders. The injury due to this enemy alone is difficult to ascertain and is a problem to be solved in the future.

The caterpillars feeding on lac insects normally containing the red lac dye ultimately give rise to moths coloured pink. Imms and Chatterjee[1] have given a colour picture of the moth, *E. amabilis*. There could be other factors besides the lac dye which may contribute to the depth of colour shown by the moth. Specimens of moth from Mysore were deeper in shade than that illustrated by Imms and Chatterjee. Figure 7a gives a natural size picture of this moth. In an experimental inoculation of the species, *Lakshadia communis*, on *Guazuma tomentosa*, at Bangalore, the third larval stage was severely attacked by the chalcid *Erencyrtus dewitzii*. A caterpillar of *E. amabilis* feeding upon such food got little lac dye and the moth reared from it was pale yellow with just a tinge of pink at the edge of the markings. This abnormal pale moth looked quite different in colour and is shown as Fig. 7b; it contrasts with the normal red specimen, Fig. 7a. It confirms the role of the red dye in the food in producing the deep colouration of the normal moth Fig. 7a.

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## ESSENTIAL FATTY NUTRIENTS IN DIETS AND THEIR EFFECTS ON HEALTH, AND ON LIVER AND HEART DEGENERATION IN MICE

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Diet is one of the important factors in coronary heart disease[1] and liver degeneration.[2] Through short period experiment the fats in diets have been found to cause many ailments.[3-4] Peacock[5] has elucidated the effects of dietary fatty materials on the animal systems. In the present investigation, with long-term animal experiments the stable fat of the diet containing essential fatty acids (EFA), tocopherols, and mixture of ascorbic and citric acids, has been shown to play crucial roles on these phases of ailments.

The short-period experiments [3-5] do not reveal the cumulative effects of fats and the allied substances. The present series of experiments were, therefore, extended over the whole life span of the healthy and genetically pure male $C_3H$ mice (40 per series). All precautions were taken to eliminate causes of diarrhoea, external infections, sudden shock and panic. The mice were housed in cages having twenty in each. The hydrogenated cottonseed oil blended with 8% original cottonseed oil, was obtained from a commercial firm. One batch of cottonseed oil was heated at 180°C. with metals (cast iron, aluminium, stainless steel, monel and copper) for about four hours for establishing the aggregate effects of cooking vessels. The oil was oxidized to one per cent peroxide contents by bubbling oxygen in presence of visible

light. In order to maintain homogeneity in oils used, required quantities of cottonseed oil for all experiments were purchased at one time and stored below 0°C. The weekly diet was prepared with a fixed composition (experimental fat, 17.5; crude casein, 21.7; sucrose, 10.1; glucose, 14.1; starch, 17.1; brewer's yeast, 8.2; alfalfa meal, 4.1; salt mixture, 4.1; cod liver oil, 2.0 and wheat germ oil, 1.1%), stored below 0°C., fed *ad libitum* and weekly growth records were maintained. Within a month by gradual increase the mice were made accustomed to 1.0% peroxidized fat. However, these mice started dying from the third month and within six months all died due to heart failure. The hydrogenated cottonseed oil fed mice exhibited sound health, those fed on plain cottonseed oil medium health, and those fed on heated cottonseed oil, stunted growth. The mice were killed under ether and opened for observations.

Results are summarised in Table 1. Evidently, the stability of tocopherols in dietary and metabolic fats, plays the main role in the health of mice. The peroxidized fats destroyed tocopherols, resulting in faulty fat metabolism[3] and heart failure. The heated fats with unstable tocopherols[6] caused damage to both heart and liver. Cottonseed oil was not as stable as the hydrogenated one and the hydrogenated oil containing mixture of ascorbic and citric acids. The latter samples with stable fats and tocopherols did not affect heart, but the liver cell proliferation was very severe in one and not so much in the other (Table 1). It seems that tocopherols and fats (with EFA) stabilize each other *in vivo* as *in vitro*[7] and maintain uniform growth in all organs of mice, except liver having uncontrolled growth of cells. Probably, some inhibition of such excess proliferation, by ascorbic and citric acids, may be related to enhancement of tocopherol efficiency as biological antioxidant with cellular control mechanism preserved.

TABLE 1.—TOCOPHEROLS IN DIETARY FATS AND HEALTH OF $C_3H$ MICE.

| Experimental fats used in the diets | Tocopherol contents in fats γ */g. | Heart failure | | Liver cell prolifer-ation | |
|---|---|---|---|---|---|
| | | Incidence % | Duration (months) | % animals having initiation | Duration |
| HCO* | 824.0 | 0.0 | 12½ | 92.0 | 12½ |
| HCO+Ascorbic & citric acids** | 938.0 | 0.0 | 12½ | 92.0 | 12½ |
| CO | 1120.0 | 15.0 | 12½ | 45.0 | 12½ |
| Heated CO | 135.0 | 37.0 | 12½ | 29.0 | 12½ |
| CO with 1.0% peroxides by weight of CO | 0.0 | 100.0 | 6.0 | 0.0 | 6.0 |

* HCO ; Hydrogenated Cottonseed oil; CO : Cottonseed oil; microgram: γ.

** 60 mg. Ascorbic and 150 mg. citric acid per 100g. of diet.

In another series of preliminary experiments, excess tocopherols, eight times in amounts, in comparison to those present in hydrogenated cottonseed oil (HCO) isolated from cottonseed oil (CO) and added to hydrogenated cottonseed oil, caused stunted growth in mice. It seems that excess tocopherols initiate autoxidation[7] *in vivo* and deteriorate EFA[1,7,9] producing faulty fat metabolism.[3,10]

These findings indicate interesting facets of aging and ailing processes, although extreme caution must be employed in translating data from one species to another. Tocopherols as biological antioxidants *in vivo* with excess becoming prooxidants, as *in vitro*[7], may be influenced[1,3] by choline, lecithin, phosphatides, amines, inositol and pyridoxins, amidst[3] glutathione, ascorbic, citric and phosphoric acids in correcting the faulty fat metabolism[3] in which EFA, vitamins A and pantothenic acid, may have significant roles.[1,8] Such influence on tocopherols is governed by the antioxidant and synergistic actions of these substances[1,3,10,11] affecting the autoxidation behaviour of EFA and vitamin A.[1,11] Pantothenic acid helps metabolism of fats[12] in conjunction with EFA.[3,13]

The proportionate amounts of foods[8,14,15] (balanced diet in particular) are important in the maintenance of health free of ailments. Consequently, dietary bulk and ratio of carbohydrate, protein, and fat with considerations for ingestion[8] of the above and other nutrients, physiologically active agents and nutrient-antagonists (say, in the form of preservatives, flavours, colours, additives and luxurious uses of plastic and polymer[8] utensils in foods and food preparations) may reflect the ailing and aging mechanisms after due reservations for heredity, sex life's stress and strain. These points are discussed in the foregoing sections, in order to present a coherent picture of the field and its essentials.

Now considering the present day luxurious and lazy approach to ready-made foods with many contaminants,[8] a prediction should be made for stimulating people's cautiousness and also for further investigation. Probably, by 1970, man may present many medical problems, because man's system takes more than 20 years to exhibit any abnormal effects.[8,16]

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