Foodstuff	Thiamin				Nicotinic Acid			
	Enzymic hydrolysis		Autolysis with water		Enzymic hydrolysis		Autolysis with water	
	μg./g.	% recovery	μg./g.	% recovery	μg./g.	% recovery	μg./g.	% recovery
Yeast								
Brewer's	97·9	92·3	96·2	96·0	436·7	95·0	436·7	95·0
Torula (grown on molasses medium) .. .	26·0	97·8	21·0	104·0	215·93	93·0	—	—
Nuts								
Groundnut (*Arachis hypogæa*) (ether extract) ..	16·7	99·0	—	—	—	—	—	—
Animal Tissues								
Sheep, liver	5·8	86·7	5·2	88·8	168·5	100·0	168·5	100·0
,, heart	4·8	95·2	3·8	81·0	37·0	—	35·1	—
Cereals								
White maize (*Zea mays*) ..	4·2	101·0	4·7	86·4	11·8	105·0	9·8	92·8
Wheat (*Triticum vulgare*) ..	3·9	89·6	3·9	89·6	23·8	96·8	—	—
Pulses								
Bengal gram (*Cicer arietinum*)	4·9	87·0	—	—	20·0	93·3	—	—
Red gram (*Cajanus indicus*)	3·8	102·0	3·5	96·0	23·0	86·0	—	—

lished results) and another portion was taken for the nicotinic acid test, after being heated in a boiling water-bath for 30-40 minutes to hydrolyse nicotinic amide to nicotinic acid. The latter was brought to pH 7·0, and nicotinic acid was estimated according to Swaminathan's method.[15] The results of tests carried out with various foods are shown in the table.

The figures for percentage recovery given in the table show that enzyme digestion is the best means of liberating and extracting the two vitamins. Further, the method is also effective in reducing the interference by extraneous substances to a minimum. In the case of fresh foods and yeast preparations, dried in the sun, mere autolysis with water at pH 6-7 was found to liberate both thiamin and nicotinic acid from their biological combinations (see table). However, the use of the enzyme preparation is recommended to ensure complete liberation of the vitamins from all types of foods.

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Indian Research Fund Association,
Coonoor, KAMALA BHAGVAT.
March 8, 1943.

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TOXICOLOGY OF FOOD COLOURS AND THE NEED FOR THEIR DETAILED PHARMACOLOGICAL EXAMINATION

VARIOUS types of colouring matters, from coal-tar products or from vegetable and mineral sources, have been used by manufacturers of food and dairy products for a long time in Europe and America. Addition of a colour or a mixture of colours to render processed food preparations or beverages 'attractive' and sometimes, also to conceal damage, adulteration or inferior quality is a very common procedure in industrial countries, more particularly marked in America during the last two decades. The problem is slowly assuming importance in India in connection with the proposed colouring of 'vegetable ghee' (hydrogenated vegetable fat) to make it look 'distinctive' from cow or buffalo ghee (clarified butter).

Coal-tar dyes being most readily available during the pre-war days at reasonable prices naturally attracted more attention in India, as elsewhere. As ordinarily manufactured for textile or other industrial purposes, dyes often contain impurities (*e.g.*, traces of arsenic from nitric or sulphuric acid almost invariably used in dye manufacture, lead and copper from utensils employed, harmful organic compounds and intermediates produced by side reactions, etc.), some of which are harmless, whereas others are toxic. These impurities may not detract from the value of the dyes for industrial use, but they would be highly objectionable in a substance designed for human consumption. The Food and Drug Administration of the United States Department of Agriculture[1] have, after careful pharmacological and toxicological examination extending for years, adopted a list of *fifteen* oil- or water-soluble dyes of various shades for purposes of certification to the trade as 'harmless' (in certain concentrations) for human consumption. No dyes outside this list are permitted to be employed until the Federal Administration is

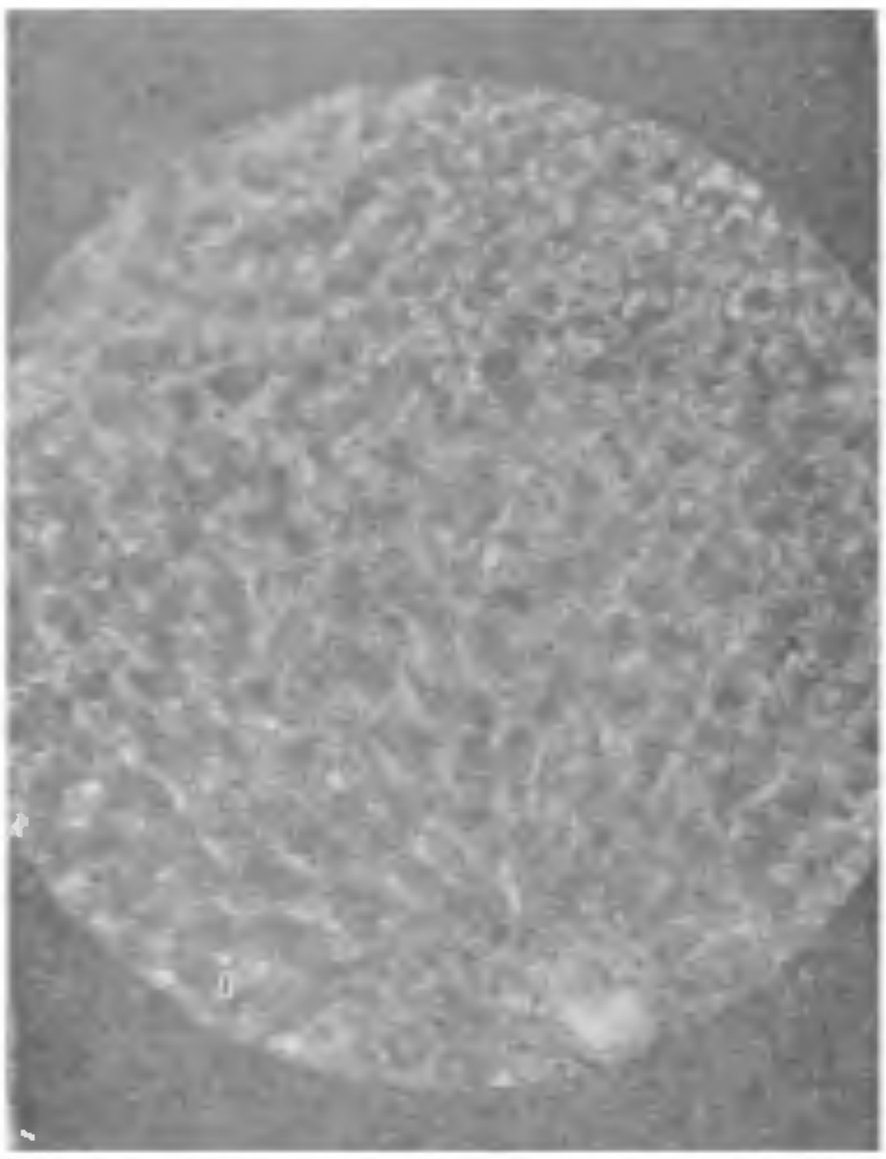

FIG. 1

Liver of a rat, ♂ 210 gm. fed daily with Oil Orange E in arachis oil (approx. 0·4 % dye concentration) well marked degeneration of liver cells (periportal): areas of healthy liver cells round the central vein. The black spots to the left of the vein are eosinophilic infiltration.

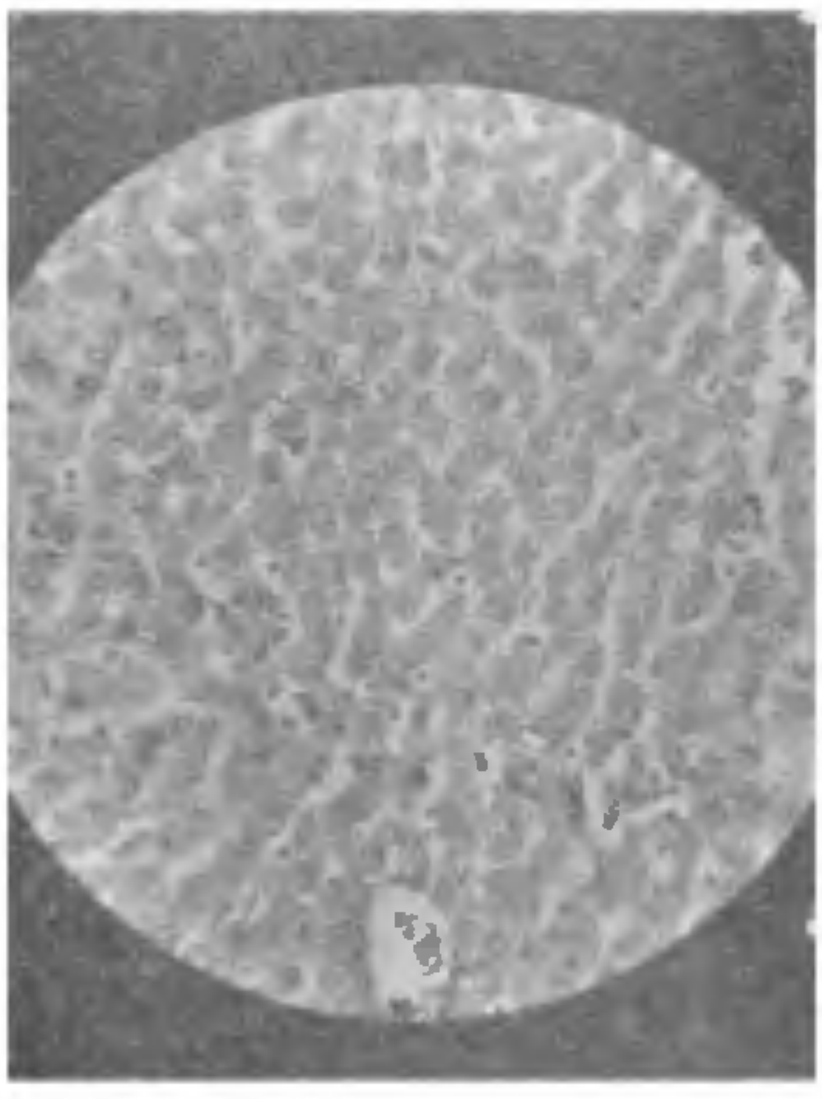

FIG. 2

Liver of a control rat on identical diet minus Oil Orange E. No pathological change is noticed.

satisfied about its purity and absolute safety after a critical and elaborate study of the chemical, pharmacological and toxicological properties of the dye.

During the last two years, the Biochemical Standardisation Laboratory was afforded the opportunity of investigating the pharmacological and toxicological properties of two food colours, one of coal-tar origin (Oil Orange E or Benzene-azo-β-naphthol), and another of vegetable origin, 'Ratanjot' (*Onosma echiodes*). 'Chronic' feeding experiments on white rats extending for 2-4 months were carried out with the dyes in different concentrations (colour intensity readings recorded by means of the Lovibond Tintometer—1938 model), following, in essential, details recommended by Hesse.[2] *Post-mortem* examinations of liver, kidney and heart were made at various stages of the feeding experiments and histopathological changes recorded in permanently mounted paraffin sections.

Addition of 'Oil Orange E' to arachis oil in a concentration adequate to bring the Tintometer reading approximately to 30 Yellow and 30 Red Units (heated to 160-170° C. in presence of moisture as obtainable in ordinary cooking) when fed to white rats in a dosage of about 0·5 c.c. per rat (average weight, 150-200 gm.) every day for 60 days produced loss of weight and a demonstrable pathological change in the liver (see microphotographs). In a less extensive study on rats, Basu[3] has also recorded loss of weight after 8 weeks' continuous feeding, associated with liver and kidney congestion or degeneration. We could not demonstrate any pathological change in the kidney and heart simultaneously with the liver changes.

Organic colouring matters of natural origin such as vegetable colours are usually, relatively speaking, harmless in themselves, or free from harmful impurities. In this class of colouring matters also, it appears desirable to exercise some vigilence, particularly in regard ot the amount of dye to be used daily and the concentration in which it is likely to be ingested along with edible fats. Our experience with 'Ratanjot' as a colouring agent seems to indicate that, in fairly high concentrations and under certain conditions of administration, even a vegetable dye can bring about toxic manifestations as evidenced by pathological changes in the liver and loss of weight of the experimental animals. Whether it is due to any impurity introduced into the dye in the process of extraction or to other factors is yet unknown. Details of the investigations will be published elsewhere.

The need for regular 'certification' of new food colours and the preparation of a list of 'permitted' dyes obtainable from indigenous vegetable and mineral sources (after very thorough and critical pharmacological and toxicological examination) are, therefore, of importance in the interests of consumers of coloured food and dairy products in India. This should be done preferably through a State

Organization, as in America and other European countries.

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Calcutta,
February 26, 1943.

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ACTION OF SULPHONAZIDES ON HETEROCYCLIC COMPOUNDS

IN exploring the various possible methods of synthesis of sulphanilamido derivatives of heterocyclic compounds, we came across the reaction discovered by Curtius and Rissom[1] which appeared to give access to compounds otherwise difficult to prepare. This reaction, which can be represented as follows

$$p\text{-R-C}_6\text{H}_4\cdot\text{SO}_2\text{N}_3 + \text{R'H} \rightarrow p\text{-R}\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{NH}\cdot\text{R'} + \text{N}_2$$

where R = H, Me, Cl and NH·Ac

has been extensively studied by Curtius and collaborators.[1] It has been found that in case R'H is benzene, toluene, xylene or naphthalene, the reaction proceeds as represented above, but with aniline, mono-methyl and dimethylaniline it is more complicated yielding other products also, one of them being the sulphonamide ($R.C_6H_4.SO_2NH_2$) arising from the azide. While pyridine yields a (2- or 3-substituted) benzenesulphonamido derivative, with quinoline the only crystalline product isolated is the sulphonamide corresponding to the starting azide.

We have studied, in the first instance, the action of acetsulphanilylazide[2] on thiazole, 2:4-dimethylthiazole, 2-hydroxy-4-methyl thiazole, pyridine and glyoxaline.

Thiazole and 2:4-dimethylthiazole in excess were kept boiling with acetsulphanilylazide till there was no more evolution of nitrogen (12 to 24 hours). In both the cases, the volume of gas collected corresponded roughly to one molecular equivalent. From the reaction products, the only crystalline product that could be isolated in the two cases was *p*-acetaminobenzenesulphonamide in yields of 25 and 5-10 per cent. respectively. Similarly *p*-toluenesulphonazide and 2:4-dimethylthiazole furnished only *p*-toluenesulphonamide in 75 per cent. yield. The action of acetsulphanilylazide on 2-hydroxy-4-methylthiazole led to no tangible product.

Pyridine on boiling with acetsulphanilylazide as described above furnished in poor yields an acetsulphanilamidopyridine, m.p. 280°, which appears to be the 3-substitution product (the m.p.s of the 2-, 3-, and 4-substituted products recorded in literature[3] are 224-227°, 272-275° and 252° respectively).

Glyoxaline in sharp contrast to all compounds studied so far reacted very violently with one molecular equivalent of acetsulphanilylazide at 80 to 110°; one molecular equivalent of nitrogen was evolved in less than a minute and the product obtained was a tar. When, however, the reaction was carried out in small quantities carefully regulated, one molecular equivalent of nitrogen was evolved in about thirty minutes; the product obtained was a mixture from which two crystalline compounds were isolated (with very indefinite m.p.) none of which is identical with *p*-acetaminobenzenesulphonamide. The structures of these are under investigation.

We are studying the action of the sulphonazides on various other compounds.

Haffkine Institute,
Parel, Bombay, (MISS) B. S. ALAMELA.
March 29, 1943. K. GANAPATHI.

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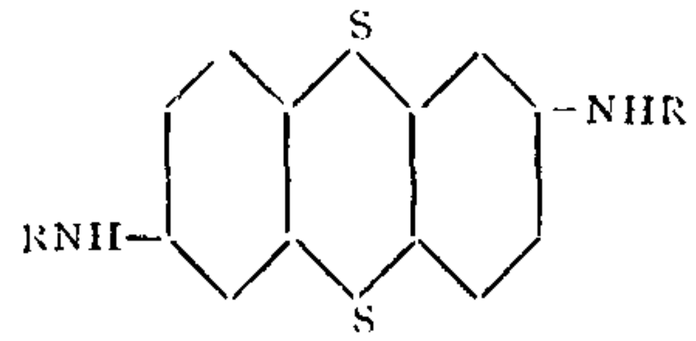
SYNTHESIS OF SULPHANILAMIDE DERIVATIVES OF THIANTHRENE

SULPHANILAMIDO compounds possessing heterocyclic rings have come into great prominence as therapeutic agents with the discovery of sulpha-pyridine (B.P. 516288), sulphathiazole, etc. The disulphanilamido derivative of 2:6-diamino-thianthrene has now been prepared.

2:6-Diacetaminothianthrene was prepared according to the method of Ray.[1] The corresponding diamine was obtained from the diacetyl compound by hydrolysis with hydrochloric acid and neutralising with alkali (yellow needles, m.p. 120°C.). *p*-Acetamino-benzene-sulphochloride reacts with 2:6-diaminothianthrene to yield the diacetyldisulphanilamide of thianthrene (I) which decomposes at 180°.



(I, R = $-SO_2\cdot C_6H_4\cdot NHAc$)

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Bangalore, P. C. GUHA.
March 12, 1943. V. M. DOKRAS.

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