

period, rats fed the supplemented diet had gained 2.5 times as much b.wt and 3.3 times as much body fat as the rats consuming equal amounts of energy from the fat-free diet. The essential fatty acid deficient rats produced more heat per unit b.wt than did the rats fed the corn oil supplemented diet. These results are in agreement with an earlier report of increased oxygen consumption⁶ in essential fatty acid deficient rats and suggest that essential fatty acid deficient rats have increased cellular thermogenesis. Rats fed the 2% corn oil supplemented diet had heavier livers than rats fed the fat-free diet, but liver particulate protein content, expressed as mg per g of liver, was unchanged (table 2). Kidney weights and protein content were not influenced by the diets.

The specific (³H)-ouabain binding in liver and kidney preparations from rats fed the fat-free diet was increased approximately 40% (table 2). This increase in (³H)-ouabain binding was specific for saturable binding since nonsaturable binding was not affected by the diet fed. The increase in specific ouabain binding observed in the liver and kidney preparations from essential fatty acid deficient rats was due to an increase in concentration of ouabain binding sites rather than to alterations in affinity for ouabain because the affinity of each binding site for ouabain (K_d value) remained unchanged. Since the livers of the fat-free group weighed less the specific ouabain binding, expressed on a total liver basis, was not significantly elevated in the rats fed the deficient diet. But specific ouabain binding to kidney preparations from the fat-free group, expressed per total kidney weight, was significantly higher than that of the 2% corn oil supplemented group. The increase in specific ouabain binding observed in the liver and kidney preparations of essential fatty acid deficient rats is consistent with an earlier study⁴ in which Na^+, K^+ -ATPase activity in brain tissue from essential fatty acid deficient

mice was elevated. The mechanism of the dietary induced alteration in ouabain binding remains to be established; however, alterations in membrane lipid composition and/or prostaglandin metabolism may be involved¹⁰. An increase in membrane associated Na^+, K^+ -ATPase enzyme units in essential fatty acid deficient rats may lead to elevated ATP turnover and thus contribute to the lowered energy efficiency observed in these animals. However, it must be emphasized that a change in the number of enzyme units may not reflect enzyme activity in the intact cell. Further studies are needed to evaluate the extent to which alterations in sodium pump activity in vivo, as well as other energy utilizing processes¹¹, contribute to increased heat production in essential fatty acid deficient rats.

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Isolation of di-N-2-propylpentyl phthalate from human urine¹

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Summary. A higher percentage of di-N-2 propylpentyl phthalate isolated from the urine of the normals and a lower quantity in the patients suffering from Eales' disease accompanied with the occurrence in patients of another compound which is also most likely a phthalate ester has been correlated with the Eales' disease.

Since its discovery by Henry Eales in 1880², a number of unsuccessful attempts have been made by various workers to find the cause of recurrent retinal hemorrhages occurring predominantly in young males^{3,4}, a condition that has since come to be called as Eales' disease. So far, no definite preventive or curative treatment is available. In our laboratories, we have been attempting to find the cause of this disease, and report our findings.

Ether extracts of urine from the patients and normal individuals were analysed on TLC plates when 2 spots having $R_f \sim 0.9$ and ~ 0.34 were obtained. The concentration of the compounds was diagnostic of the disease, the compound having $R_f \sim 0.34$ occurring in higher concentrations in the patients and the compound with $R_f \sim 0.9$ existing in a higher concentration in the normals (figures 1 and 2). While we have isolated and characterized the product $R_f \sim 0.9$, the compound having $R_f \sim 0.34$ is still under investigation.

Material and Methods. Collection and extraction of urine. Collection of 24-h urine from 27 patients between 20 and

40 years (all males) and 17 normal individuals (all males) of the same age group were made under toluene and were kept refrigerated. All medications were stopped 2 days prior to the collection of urine of the patients under study. Peroxide-free, freshly distilled 30 l of ether was used for the extraction of 15 l of urine each from the patients and normals. The ether extracts on distillation yielded oily coloured products which were fractionated on alumina column for purification.

Chromatographic procedures. TLC plates were run, using benzene-methanol (3.5%) mixture, and developed with Follin's reagent⁵ in ammonia tank. Purification of the ether extracts was made on alumina column using benzene-methanol (2%).

Results and discussion. The eluates obtained after the column fractionation of the urinary extracts of the normals yielded on evaporation an oil (155 mg, $R_f \sim 0.9$) which was found to be homogeneous on TLC and gas chromatogram. Corresponding eluates from the patients extract yielded

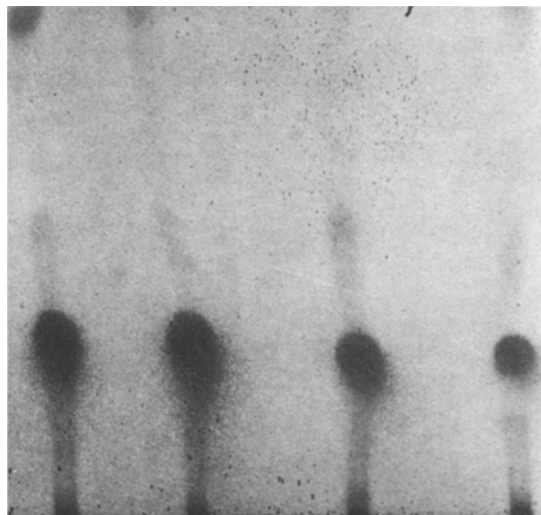


Fig. 1. TLC picture of urinary extracts of 4 different patients of primary Eales' disease.

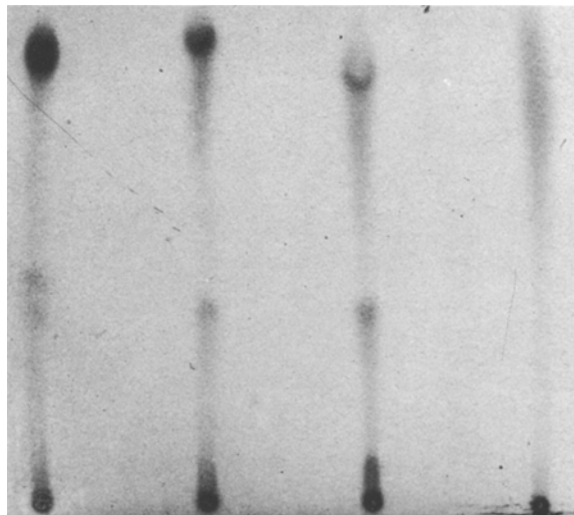
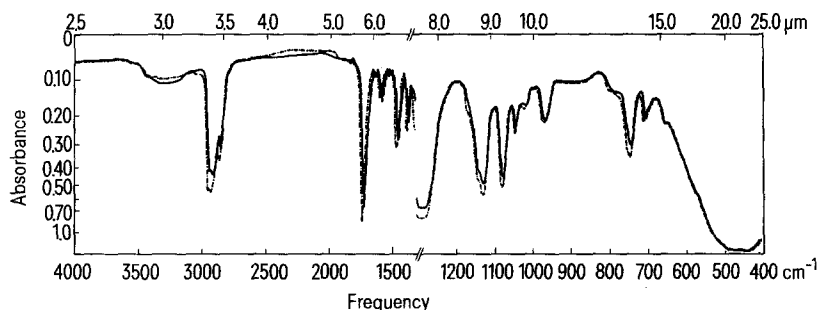


Fig. 2. TLC picture of urinary extracts of 4 different normal individuals.

Fig. 3. Comparison of the spectrum of human natural product (-----) with the same compound isolated from *Cryptocarya amygdalina* (———).



only 28 mg of this oil. Repeated efforts to obtain it in a crystalline form were unsuccessful.

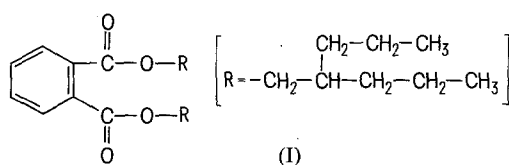
The oil analyzed as $C_{24}H_{38}O_4$; $\eta_D^{25} 1.4795$ and its UV-spectrum showed $\lambda_{max}^{MeOH} 234.277 \text{ nm}$ ($\log \epsilon 3.9, 3.1$) suggesting a conjugated benzenoid chromophore.

The IR-spectrum did not show OH absorption bands. However, prominent bands were observed at 1730 (ester), 1603 (aromatic) and 743 cm^{-1} (ortho-disubstituted benzene) revealing the presence of benzenoid diester in the molecule.

The NMR-spectrum ($CDCl_3$, 60 Hz) of the oil showed a complex multiplet centered at 2.38τ (suggesting 4 Ar.H) a doublet at 5.85τ (suggesting 4H, 2 OCH_2), a broad multiplet centered at 8.76τ (18H, methylene and methines) and another multiplet at 9.10τ (12H, 4 methyls). The sharp doublet at 5.85τ (J, 5.0 Hz) should be assigned to methylenes attached to methines and bearing oxygen functions. The mass spectrum of the compound was essentially similar to those reported for phthalic esters⁶.

On hydrolysis with 5% alcoholic NaOH, the compound yielded phthalic acid and an alcohol which analyzed as $C_8H_{18}O$ and exhibited spectral properties similar to 2-propylpentanol.

On the basis of the above evidence, the most probable structure of the compound is di-N-2-propylpentyl phthalate (I).



Its identification was further established by its chemical synthesis. 2-propylpentanol on condensation with phthalic anhydride yielded a product identical with the natural one in its spectral data. The isolation of this compound also from a plant *Cryptocarya amygdalina*⁷ will be discussed elsewhere. Identical structures for these have been established by co-TLC and comparison of the IR-spectra (figure 3). Further, this compound has been found to be present in the normals. However, it is not found in any appreciable amount in the patients with Eales' disease. This fact accompanied with the formation of the compound $R_f \sim 0.34$ in the patients might well be associated with the Eales' disease. Preliminary spectral studies of the compound having $R_f \sim 0.34$ point to its being also a phthalate ester.

Special precautions were taken to rule out possible contamination of phthalate ester from plastic wares⁸ by completely avoiding the use of such vessels during experiments and using thoroughly purified solvents and washed alumina. A control run under parallel conditions, replacing urine with water, did not show the presence of any phthalate ester.

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