

On partition of the other extracts between 80 per cent methanol and heptane, the methanol fraction was several times as active as the heptane fraction, though most of the lipid passed into the latter. By concentrating the active constituents in this way, preparations with activities 15–150 units/mgm. were obtained. The methanol fractions were subjected to chromatography on silica gel, eluting with a graded mixture of petroleum ether containing an increasing proportion of diethyl ether, and finally methanol. This procedure demonstrated the presence of four active substances. One of these was eluted by 25–75 per cent diethyl ether in petroleum ether and could be further purified by passage from ether into 2.6 per cent aqueous sodium bicarbonate. It appeared to consist of free acid.

While it would be speculative to propose that these oxytocic lipids play a part in parturition, it is clearly of interest that they are present in human amniotic fluid, and are capable of stimulating human uterine muscle.

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Tyrosine Metabolism in Melanoma

THE urine in patients with generalized melanoma contains substances which cause its dark colour when exposed to air—urinary melanogens^{1,2}, of which there are two types³: (1) nitrogen melanogens, insoluble in ether or ethylacetate, giving a positive Thormählen test^{4–6}—indole melanogens, as they are called, and (2) non-nitrogen melanogens, soluble in ether or ethylacetate, giving a negative Thormählen test—phenol melanogens.

In our preliminary reports⁷ we characterized phenol melanogens as a mixture of phenolic acids of an unknown constitution. We identified one of them as the homovanillic acid (HVA), the 3-methoxy-4-hydroxyphenylacetic acid⁸.

A quantitative method for estimation of HVA was worked out⁹ based on separation of HVA from ethylacetate extracts of urine, using paper chromatography⁹. The coloured spots obtained by detection of HVA with diazotized *p*-nitroaniline were cut out from the chromatograms, eluted and measured photocolourimetrically.

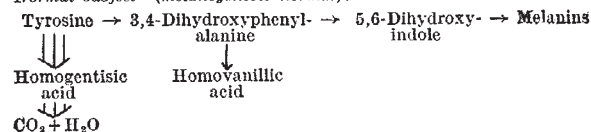
Using this method HVA excretion in the urine of patients with melanoma (metastases) was found to be 3–4 times greater than in normal people. After oral application of tyrosine, an increased urinary excretion of HVA occurred in these patients, whereas in normal health it was not changed⁸.

Similar results were also obtained with indole melanogens. Concentration in the urine we estimated using a photocolourimetric modification of the Thormählen reaction according to Pechan¹⁰. It was found that after oral application of tyrosine an increased urinary excretion of indole melanogens occurred in melanoma patients^{8,9}.

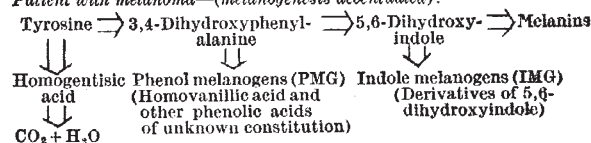
Because HVA has recently been found to be a natural metabolite of endogenous 3,4-dihydroxyphenylalanine in normal people¹¹ and indole melano-

gens are derivatives of 5,6-dihydroxyindole^{5,6}, our findings appear to be in agreement with the well-known Raper–Mason scheme for melanogenesis:

Normal subject—(melanogenesis normal):



Patient with melanoma—(melanogenesis accentuated):



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Interrelation between Tryptophan and Nicotinic Acid in Human Viral Hepatitis

CONTINUING the chromatographic work carried out on tryptophan–nicotinic acid metabolism on urine of mice affected with experimental *MHV-3* virus¹, we have investigated the effect of viral hepatitis on the relationship between tryptophan and nicotinic acid in man.

Samples of urine were collected over a 24-hr. period from normal subjects, from twenty-four subjects affected with viral hepatitis at various stages of the disease and from nine subjects who had recovered either recently or some time before. For chromatographic analysis of the urinary metabolites involved in the tryptophan–nicotinic acid system Dalglish's technique² was used. Identification of the compounds was carried out by examination of the fluorescence by ultra-violet light (3660 Å.) and by means of the characteristic colour reactions.

Of the 24 cases still suffering from the disease, in 22 we found kynurenine, in 17, 3-hydroxykynurenine, in 10, 3-hydroxyanthranilic acid, and in 3 cases anthranilic acid. Frequently, acetyl-kynurenine (15 cases), kynurenine and xanthurenine acids appeared. It must be realized that various chromatographic tests were carried out on different days for each patient, and these tests did not always give results which were perfectly identical. Usually the chromatographic pattern presented the greatest metabolic alterations at the time when the disease reached its maximum.

From the studies carried out on 9 patients who had recovered for a few months, the chromatographic