EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are summaries of the proceedings of the 12th meeting of the Experimental Biology Group (EBG) held on 24 April 1964:

USES OF 'THOROTRAST' IN EXPERIMENTAL STUDIES ON INVERTEBRATES

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Thorotrast is a stabilized colloidal suspension of thorium dioxide prepared by the firm of Testagar & Co., Detroit, USA. It is extremely radio-opaque, chemically unreactive and is a low-grade α-emitter. Its original use was in clinical hepatolienography,[6] but it was later also applied to arteriography.[1] However, as thorium particles are never excreted, their radioactivity eventually causes serious disorders which may ultimately lead to the death of the patient,[7] so that thorotrast had to be abandoned for medical purposes. It is nevertheless an invaluable tool in research on the vascular and reticuloendothelial systems of vertebrates other than man.[5]

No such studies on invertebrate animals appear to have been published despite the suitability of thorotrast for the radiographic examination of blood-movements, vascular anatomy, movements of coelomic fluid, the elimination of foreign particles from the body, and numerous other phenomena. indeed, in studying the movements of the haemolymph in gastropoda, thorotrast is the *only* radio-opaque dye I have found even remotely suitable, for in animals with open vascular systems such a large dose of dye must be injected that clinical preparations such as 'urografin' and 'hypaque' cause the rapid death of the animal. The toxicity of thorotrast is so low, on the other hand, that a dose of one-tenth of the total body weight may be injected into a gastropod without stimulating serious reactions.

This dye has now been used to study the movements of haemolymph during retraction and expansion in the intertidal sandy-beach snail, *Bullia* (results in press). The animal is suspended in front of a vertical X-ray plate and thorotrast injected into the pedal sinus through a fine hypodermic needle. Retraction into the shell can be stimulated at any stage by pinching the animal behind the head while the film is exposed by means of a horizontally-directed X-ray tube. An exposure of 0·03 seconds at 200 mA and 66 kv. at a focus-film distance of 100 cm. is sufficient to pass through both thicknesses of shell as well as the living tissues, yet give a well-contrasted thorotrast shadow on the resulting radiograph. The route taken by haemolymph leaving the pedal sinus can thus be clearly seen. Other vascular problems in *Bullia* are being elucidated by similar methods.

These snails continue to thrive in the laboratory for an indefinite period after the injection of thorotrast and thus provide an ideal opportunity for studying the elimination of the dye from the body, for the thorium particles are not stored as in vertebrates but are phagocytosed by the haemocytes which then migrate through definite routes to the exterior of the snail. The gross elimination of the thorium may be studied by taking radiographs at regular intervals, while the more detailed distribution of the particles may be assessed by examining sections by means of the method of combined oil immersion, dark-ground and phase-contrast described by Baxter.[2] Under these conditions the thorium particles stand out in brilliant and opaque white against the translucent and subdued coloration of the tissues.

Thorotrast has also been used to solve the difficult problem of the origin of the jets of water which spring from the foot on retraction of the snail.[3, 4] The sea-water normally contained in the mantle cavity, the free space, or the proboscis sheath can be replaced with thorotrast by means of an arterial catheter; the opacity of the jets in radiographs exposed during retraction shows conclusively which spaces have contributed to them.

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THE TWENTY-FOUR-HOUR URINARY OUTPUT OF NON-IONIC OSMOLES; AN INDIVIDUAL PHYSIOLOGIC CONSTANT

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During the course of a study concerning the ionization of inorganic salts in urine, we found that (*i*) 90% - 95% of the ionic constituents of normal urines were univalent, and (*ii*) urinary osmolality and ionic strength were highly correlated. In as much as the osmolality of any given urine is a measure of both its ionic and non-ionic particulate concentrations, these findings suggest a constant ratio of ionic to non-ionic osmotically active particles in normal urines.

The 24-hour urinary output of creatinine in any individual is fairly constant. We have shown elsewhere that urinary creatinine and osmolar concentrations are highly correlated. It follows that the 24-hour excretion of total, ionic and non-ionic osmotically active urinary particles might also be expected to be constant.

Urinary specific conductivity, like osmolality, is highly correlated with urinary ionic strength; measurement of both these parameters permits a fairly accurate estimate of ionic strength in any normal urine. As most of the ionic constituents are univalent, the ionic component of the total urinary osmoles is readily obtained. The non-ionic osmolar content is got by difference.

An alternative method of measurement of non-ionic osmolar concentration is by comparison of the dielectric constant of the urine with that of a salt solution of identical specific conductivity. This is technically more difficult.

In 109 normal subjects, non-ionic osmoles constituted approximately 40% of the total 24-hour urinary osmolar output. In a few subjects studied for weeks in a metabolic ward, the coefficient of variation of the 24-hour output of non-ionic osmoles was found to be slightly less than that of the total osmoles, and approximately the same as that of creatinine.

THE USE OF CARBOWAX 4000 IN THE HOMOGENIZING MEDIUM FOR THE EXTRACTION OF ACTIVE ENZYMES FROM GRAPE BERRIES

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In a study on the biosynthesis of organic acids through the fixation of carbon dioxide by an enzyme extract from grape berries, it was found that the addition of cysteine and carbowax 4000 to the tissue before homogenization was necessary for the extraction of active enzymes. Maximum activity was obtained by using one part by weight of carbowax 4000 to ten parts berry homogenate.

CONGENITAL ERYTHROPOIETIC PORPHYRIA*

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Congenital erythropoietic porphyria is a rare, recessively inherited, disease which appears to involve an enzyme required for the synthesis of haem. A patient with this condition was recently admitted to the dermatology wards of Groote Schuur Hospital, and certain investigations performed are reported here. The communication concerns only the disturbance of porphyrin metabolism and avoids, as far as possible, reference to the clinical condition.

The excretion of porphyrin was greatly increased: (Urine: uroporphyrin 9·4 mg./day; coproporphyrin 2·3 mg./day. Faeces: coproporphyrin 0·95 mg./G dry weight; protoporphyrin 0·07 mg./G dry weight.)

The patient maintained that her condition had been present from an early age; it was therefore suspected that she might have the congenital erythropoietic variety of porphyria. Bone marrow was aspirated from the iliac crest; ultraviolet (UV) microscopic examination of an unstained preparation showed red porphyrin fluorescence in many nucleated cells. Identical areas of a marrow smear were photographed unstained using the UV fluorescence microscope and, after staining, rephotographed using tungsten light. All fluorocytes were identified as late normoblasts and, of 23 such cells present in one area examined, 16 fluorescenced red.

Haematological investigations were performed, Anaemia was absent (haemoglobin 13·5 G/100 ml.). Red cells in peripheral blood were morphologically normal. There was only slight evidence of erythroid hyperplasia in the bone marrow. Reticulocyte counts varied from 1 - 6%. The half-life of red cells labelled with ^{51}Cr was 29 days.

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The liver did not fluoresce in UV light and the histological appearance was normal. There was, however, some chemicopathological evidence of liver disease. Liver tissue contained 29 μg. of porphyrin per gram wet weight.

Porphyrin from urine and faeces was examined in detail by column and paper chromatography and by electrophoresis. The faecal porphyrin was almost entirely coproporphyrin, of which 84% was isomer type I. Urine uroporphyrin was 94% type I, as also was urine coproporphyrin. Uroporphyrin methyl ester was crystallized and melted over the range 291 - 293°C. The excretion of 7-carboxyl porphyrin in the urine was only 10% of 8-carboxyl (uroporphyrin) excretion.

This patient is excreting increased amounts of porphyrin of the isomer I type, and this finding is typical of congenital erythropoietic porphyria. This is interpreted as a failure to convert porphobilinogen to uroporphyrinogen III because of an enzyme defect, probably uroporphyrinogen isomerase. However, the normal haematological status suggests that the patient is well able to synthesize the 300 mg. of type III porphyrin required daily for haem synthesis. As she is excreting only about 30 mg. of type I porphyrin daily, the enzyme defect appears to be mild compared with most cases described in the literature.

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