

Labelling of Rose Bengal with Iodine-131 by Radioactive Exchange

THE most widely used test for the chromoexcretive ability of the polygonal liver cells is the clearance test with rose bengal. The scope of the method was increased considerably by Taplin *et al.*^{1,2} who introduced the use of labelled rose bengal. The test achieved great diagnostic value in cases of obstructional and toxic damage. We have been able to improve the method for preparing rose bengal labelled with iodine-131.

The iodination of tetrachlorofluorescein is the normal method used for labelling rose bengal (tetrachlorotetraiodofluorescein). The iodination is carried with iodine in alkaline solution with iodine chloride³. The first method is inconvenient because of low yield and the second because it requires radioactive iodine chloride. We therefore applied the method of iodine exchange which we employed successfully for labelling of roentgen contrast media⁴. The method proved satisfactory for rose bengal as well. Yields of radioactivity up to 80 per cent were obtained.

For example 1 gm. of rose bengal (Bayer Lachema); 24 ml. of 1N acetate buffer solution, pH 5; 0.21 mgm. potassium iodide carrier, and 8.1 ml. of potassium iodide-131 solution (31 mc.) were heated to 80°C. under reflux on a water-bath for 12 hours. Rose bengal was precipitated after cooling by the addition of concentrated hydrochloric acid to pH 1, the precipitate filtrated through a Buchner funnel by suction and washed with cold water until the volume of the filtrate was 64 ml. The filtrate contained 9 per cent of the added radioactivity. The precipitate was dissolved in a slight excess of concentrated ammonia and again precipitated, filtered and washed. The recrystallization was repeated once more. The yield of rose bengal -¹³¹I was 80 per cent with 78.8 per cent of the original radioactivity. The radioactivity was determined in liquid samples with a scintillation counter.

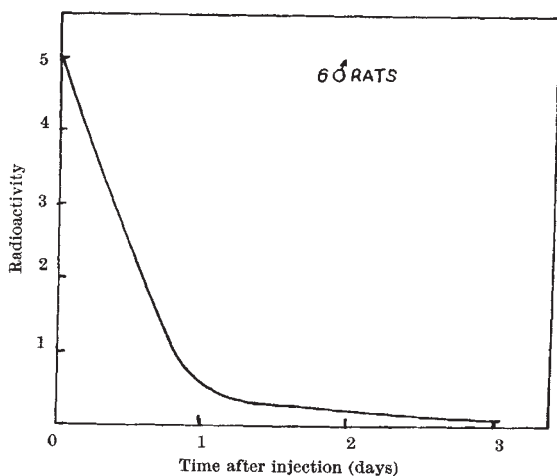


Fig. 1. Decrease of radioactivity of rose bengal-¹³¹I after intravenous injection.

The biological properties of the product obtained by this method were tested. 6 rabbits were injected intravenously with 20 µc. of rose bengal -¹³¹I, the radioactivity of the thyroid was 8 per cent higher than the activity measured over the thigh under identical conditions. In a group of 6 rats the total decrease of radioactivity was determined after intravenous injection of 5 µc. of rose bengal -¹³¹I per

200 gm. The results are given in Fig. 1. For this experiment the rats were put in a circular cage around which 6 Geiger-Müller counters were placed in order to measure the decrease in radioactivity. Counts were taken 0.5, 18, 24, 48 and 72 hours after injection. Fig. 1 shows that practically all the radioactivity is excreted within 72 hours.

The diagnostic applicability of the compound was tested first on 24 rabbits suffering from carbon tetrachloride poisoning and positive results were obtained within 24 hours with doses as low as 20 µgm./1 gm. given orally, and secondly on a group of 6 rabbits with a ligation of the common bile duct. The results with clinical cases were satisfactory.

J. LIEBSTER
O. ANDRYSEK

Radioisotope Laboratory, Biological Institute,
Czechoslovak Academy of Science
and Biophysical Institute,
Medical School, Charles University,
Prague.

¹ Lowenstein, G. M., *Proc. Soc. Exp. Biol. Med.*, **93**, 377 (1956).

² Taplin, G. V., Meredith, O. M., Jr., and Kade, H., *J. Lab. Clin. Med.*, **45**, 665 (1955).

³ Renault, H., Jacquier, C., Jemmet, H., and Jammet, J., *Proc. First Unesco Int. Conf. Paris*, 1957, **3**, 719.

⁴ Liebster, J., Kácl, J., and Babický, A., *Nature*, **183**, 1474 (1959).

BIOLOGY

Immunological Distances among some Gallinaceous Birds

INFORMATION on phylogeny and systematics can be obtained from immunological relationships since red-cell antigens are gene-determined. To measure the immunological relationships between two species the following formula was used:

$$I.D. = \frac{1}{\sqrt{\frac{Oa \cdot Ob}{Ea \cdot Eb}}}$$

where *Oa* and *Ob* are titres with homologous cells (using anti-*a* and anti-*b* sera), and *Ea* and *Eb* are titres with heterologous ones. This value is named 'immunological distance', because it will tend to increase as the biochemical relationship, that is the proportion of common antigens, decreases¹.

An immunological analysis of red-cell antigens of five species of gallinaceous birds has been carried out. These species were: domestic fowl (*Gallus domesticus*), guinea hen (*Numida meleagris*), turkey (*Meleagris gallopavo*), japanese quail (*Coturnix coturnix*) and ring-necked pheasant (*Phasianus colchicus*). Titres are given in Table 1. The immunological technique has

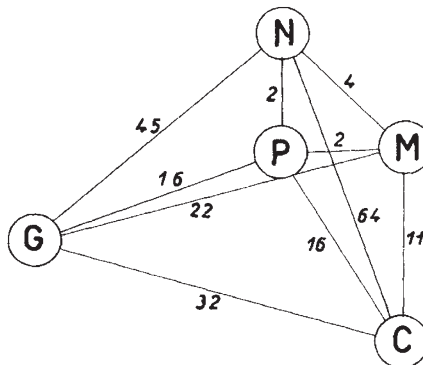


Fig. 1. Immunological distances among *Gallus* (G), *Coturnix* (C), *Phasianus* (P), *Meleagris* (M) and *Numida* (N).