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Biological half-lives of [4-^{14}C]testosterone and some of its esters after injection into the rat

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Biological half-lives for ^{14}C from labelled testosterone in muscle and whole body of rats have been measured after intramuscular injection of [4-^{14}C]testosterone and its lower esters (formate to valerate). A relation has been observed between ethyl oleate–water distribution coefficients, biological half-lives in the rat and "times of maximum effect" in the rat and fowl.

After intramuscular injection, testosterone has a shorter duration of biological action, than its esters. A possible reason is that the rate determining process is the transfer of the hormone from the globules of solution, at its site of injection in the muscle, to the surrounding tissues. Thus, testosterone, which would be expected to have an oil to water distribution coefficient more heavily in favour of water than the coefficients of the esters, would be transferred the more rapidly. Similarly, the differences in duration of biological action between esters would be related to their distribution coefficients. This theory is tested below by comparing the distribution coefficients between water and ethyl oleate, of testosterone and its esters, with retention times in the rat and in the muscle into which injected.

EXPERIMENTAL

Solubility determinations

Saturated solutions of the esters in ethyl oleate were prepared by percolation (James & Roberts, 1968) and assayed by measuring the absorbance of the carbonyl stretching peak at 1680 cm^{-1}, which had been found to be linearly related to steroid concentration. Gravimetric analysis and ultraviolet spectrophotometry were precluded by the low volatility and irrelevant absorption of the solvent.

Preparation of radioactive doses

[4-^{14}C]*Testosterone and testosterone*[4-^{14}C]*propionate* were obtained from the Radiochemical Centre, Amersham. For each preparation, 50 mg of inactive steroid was added to 50 μCi of the radioactive solution, which was carefully evaporated to dryness.

Testosterone[4-^{14}C]*formate* was prepared by heating labelled testosterone of activity 50 μCi (50 mg), with 85% formic acid (0·6 ml) at 60° for 2 h. The crystals, which separated when the hot solution was poured into cold water, were recrystallized from n-hexane.

Testosterone[4-^{14}C]*acetate, -butyrate and -valerate* were prepared by heating labelled testosterone of activity 50 μCi (50 mg) with pyridine (1·5 ml) and the appropriate acid anhydride (0·3 ml). The esters separated on pouring the hot mixture into cold water,

and were recrystallized from 95% ethanol. All melting points agreed with those quoted in the literature.

Solutions for injection were prepared by dissolving about 50 mg of the ester in 5 ml of ethyl oleate. Exact concentrations were determined by weighing.

Determination of elimination rates

Albino male rats, 250 g, were used. 0·1 ml of injection, containing 1 μCi of activity and 1 mg of steroid, was introduced into the left gluteus muscle of each rat. The syringe was weighed before and after injection to determine the precise quantity of materials used. For whole body elimination, urine and faeces were collected at 24 h intervals, and the activity remaining in the body calculated by difference. For muscle elimination, animals were killed after the required period of time and the whole leg removed. Expired air was not examined because this has been shown by Ashmore, Elliot & others (1953) to be inactive after administration of [4-^{14}C]testosterone.

Preparation of extracts for counting

Faeces were extracted as described by Martin (1966). The solution for counting was prepared by dissolving the residue in benzene and adjusting to 10 ml.

Urine was added directly to the scintillator.

Muscle. The whole upper leg was digested in 50 ml of 2N methanolic potassium hydroxide at 60° for 4 h. The solution was centrifuged, after removing the bone, and samples taken from the methanolic layer. The fat layer gave negligible counts.

Determination of Carbon-14

Hall and Cocking's scintillator (1965) (PPO–POPOP–toluene–2-ethoxyethanol) was used for faeces extracts, and PPO–POPOP–naphthalene–dioxan (Graham & Nicholls, 1959) for muscle digests and urine. 0·1 ml of sample was added to 7·5 ml of scintillator, and counted in an I.D.L. Tritium Scintillation Counter No. 6012. Internal standards were prepared by dissolving the original injections in benzene and dioxan respectively.

RESULTS AND DISCUSSION

The plots of the logarithms of radioactivity remaining against time, were linear for elimination from both whole body and muscle. First order constants were obtained by least squares analysis. Biological half-lives, the times at which only half the initial activity is retained, were calculated from the rate constants, and are given in Table 1. The variation in biological half-life, as the homologous series is ascended, is shown in Fig. 1A and B.

Miescher, Wettstein & Tschopp (1936) measured the biological responses in rat and fowl after one injection of testosterone or its esters, and their results, which were shown graphically, have been interpreted by Dorfman & Shipley (1956) as "times of maximum effect", the times at which Miescher's graphs reached a maximum. The change in Dorfman's figures, as the homologous series is ascended, is shown in Fig. 2A.

Solubilities in ethyl oleate (% w/v) are: testosterone, 0·69; formate, 5·27; acetate 3·14; propionate, 5·16; butyrate, 5·10; valerate 3·98. The logarithms of the distribution coefficients, calculated as the ratio of these solubilities to those in water (James & Roberts, 1968), are plotted against the position of the ester in the homologous series in Fig. 2B.

Figs 1A, 2A and B suggest that retention of the carbon-14 in rat is related to time of maximum effect in rat and fowl, as determined by Miescher & others (1936), and all

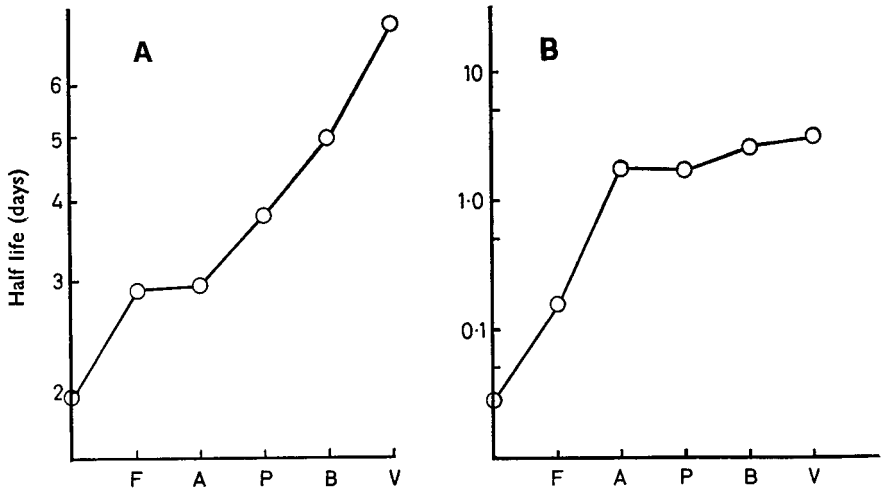

FIG. 1. Biological half-lives in A, rat and B, muscle; F, formate; A, acetate; P, propionate; B, butyrate; V, valerate.

are in turn related to the distribution coefficient. This was established more critically by plotting the logarithms of the above biological results against log distribution coefficient. Three approximately parallel straight lines were obtained, all having correlation coefficients greater than 0·95. However, the points for log biological half life in muscle were scattered (correlation coefficient = 0·71), and gave a regression line steeper than the other three lines. This is confirmed by Fig. 1B, in which the profile for half-life in muscle is different from the profiles in Figs 1A, 2A and B. It appears therefore that the rate of elimination from muscle has little connection with distribution coefficient, and therefore is not dependent on the rate of transfer from the globules of injection to the surrounding tissues. It is further evident that the rate determining step in the duration of activity of testosterone and its esters is not centred in the muscle tissue where the injection was given. This conclusion also follows from the half-lives in muscle, which are less than those in the whole rat (Table 1).

Samuels (1966) has pointed out that a distribution coefficient favouring lipids leads to concentration in fatty tissue, because the distribution coefficient is proportional to the rate of entry into fat and inversely proportional to the rate of release. Plotz & Davies (1957) have detected significant levels of progesterone in body fat, after

Table 1. *Biological half lives of carbon-14 in rat after intramuscular injection of [4-^{14}C]testosterone and its esters*

	Half life (days)	
	Muscle	Whole body
Testosterone	0·029 (10)	1·99 (3)*
Formate	0·155 (10)	2·82 (2)
Acetate	1·74 (10)	2·94 (2)
Propionate	1·63 (10)	3·75 (4)
Butyrate	2·54 (10)	4·94 (3)
Valerate	2·97 (15)	7·43 (4)

* No of animals

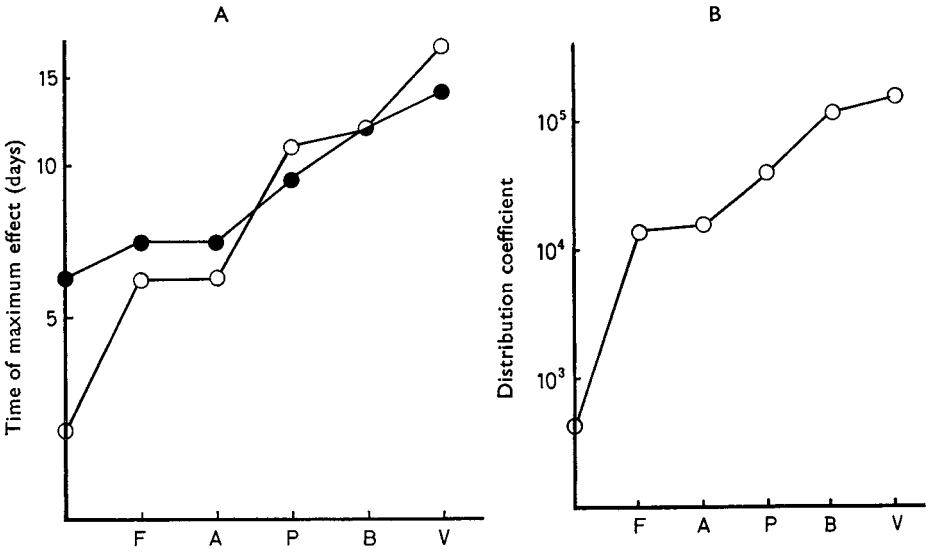

FIG. 2. A. Times of maximum effect. B. Distribution coefficients. F, formate; A, acetate; P, propionate; B, butyrate; V, valerate. ○, rat; ●, fowl.

intramuscular injection, and infer that absorption from an oily muscular depot must be fast, and that progesterone and/or its metabolites diffuse promptly from blood to fat. The results obtained in this investigation favour a similar mechanism for testosterone and its esters, and suggest that differences in times of maximum effect are a consequence of the differences between their distribution coefficients, which affect the relative rates of release from body fat.

It is probable that the rates of elimination from muscle do not correlate with distribution coefficients because ethyl oleate is absorbed at a rate similar to that for the steroids. Support for this suggestion is provided by Deanesly & Parkes (1933) who showed that appreciable quantities of olive and sesame oils are absorbed from subcutaneous tissue within 2 days of injection in rat.

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