alcohols has been established. Some saturated sterol (probably β -cholestanol) is present and a number of other sterol or sterol-like substances which form insoluble digitonides have been found.

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The Vitamin D Content of Tissues of Rats given Ergocalciferol

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In previously reported balance experiments, in which rachitic rats were given a single massive dose of 1 mg. of ergocalciferol, it was found that only about 6% was recovered in the body (Cruickshank, Kodicek & Armitage, 1953). About half of it was present in the liver, which therefore appeared to be the principal store of the absorbed vitamin. It was impracticable in that investigation to examine the distribution in all the individual organs and tissues but the possibility existed that some organs, though small in size and therefore contributing little to the total content, might nevertheless have a relatively high concentration. Subsequent work, already briefly reported (Cruickshank & Kodicek, 1952), showed that this was so and details of the experiments are now presented.

EXPERIMENTAL

The design of the experiments is shown in Table 1. The experimental procedure was that already described (Cruickshank *et al.* 1953). Groups of rachitic rats were given by mouth 1 mg. crystalline ergocalciferol/rat and the vitamin D content of various organs in the fresh state, 24 and 48 hr.

after dosing, was determined biologically on rats by the radiographic method of Bourdillon, Bruce, Fischmann & Webster (1931).

The determination of vitamin D was carried out in three series of assays, in which about 200 rats were used. The ranges of doses, in mg. of fresh material, were as follows: muscle, 100–1000; skin, 200–1000; kidneys, lungs and spleen, 15–60; brain, 125–500; adrenals, 32. Since in the previous work the whole carcass from undosed rachitic rats contained little or no vitamin D (Cruickshank *et al.* 1953), it was considered unnecessary to assay the individual organs of undosed animals with the exception of the adrenals, which when assayed were found to contain no vitamin D.

Table 1. *Design of experiments*

Each rat received 1 mg. ergocalciferol

Expt.	No. of rats	Sex	Day of killing	Tissues investigated
1	5	3 M. 2 F.	1st	Muscle, skin, kidneys
2	5	3 M. 2 F.	2nd	Muscle, lungs, spleen, kidneys, adrenals
3	6	3 M. 3 F.	2nd	Muscle, skin, brain

RESULTS

Table 2 shows the distribution of vitamin D in various organs on the first and second days after the administration of ergocalciferol. For comparison the vitamin D contents of liver and intestinal tissue found in previous experiments (Cruickshank *et al.* 1953) have been included. Although it was found that the liver contained most of the absorbed vitamin, our present results indicate that there is an appreciable amount also in muscle and skin. Each of these two tissues showed an apparent increase between the first and second days which barely reached the 5% level of significance ($P=0.03$ for muscle and 0.05 for skin). The kidneys contained an amount of the same order despite their small size. The lungs, spleen, brain and adrenals contributed little to the vitamin content of the body.

The kidneys contained a relatively high concentration of the vitamin. Thus on the second day the estimated value was 204 i.u./g., i.e. slightly though not quite significantly higher ($P=0.06$) than that found in the liver, 123 i.u./g. In lungs, spleen and adrenals the concentration was of the order of

100 i.u./g., while in muscle and skin it was relatively low and in brain negligible.

In Table 3 the sum of the vitamin contents in the individual tissues examined in the present studies (as given in Table 2) is compared with the total given by Cruickshank *et al.* (1953) for the 'carcass' (i.e. the total body minus the liver and intestines). For the rats killed on the first day after dosing, the total content of the muscle, skin and kidneys was estimated to be 620 i.u./rat, a value not significantly different from the previous estimate for the 'carcass' of 690 i.u./rat. For the rats killed on the second day, the total content of muscle, skin, kidneys, lungs, spleen, brain and adrenals was estimated to be 890 i.u./rat; this value is significantly higher ($P=0.04$) than the previous estimate, for the 'carcass', of 630 i.u./rat.

Statistical treatment

The statistical analysis follows closely that described in more detail in Part II of Cruickshank *et al.* (1953). The main features of the three series of assays are shown in Table 4.

Table 2. Vitamin D content per g. fresh weight and per whole tissue of rats given 1 mg. ergocalciferol (40 000 i.u.)

In all tables the potency per g., when less than 100 i.u., was calculated to the first decimal, otherwise to the nearest whole number. The total content in i.u., when less than 50 i.u., was rounded off to the nearest unit; otherwise to the nearest 10, except for replicate determinations which are later averaged. Data for liver and intestinal tissue from Cruickshank *et al.* (1953).

Tissue	1st day. Vitamin D					2nd day. Vitamin D				
	Expt.	Mean wt./rat (g.)	(i.u./g. fresh wt.)	Total (i.u./rat)	95% fiducial limits (%)	Expt.	Mean wt./rat (g.)	(i.u./g. fresh wt.)	Total (i.u./rat)	95% fiducial limits (%)
Liver	—	3.74	385	1440	69–144	—	4.16	123	510	67–150
Intestinal tissue	—	4.66	53.7	250	69–145	—	4.70	19.2	90	68–147
Muscle	1	13.6	18.1	250	70–142	2	13.2	34.0	449	390* 78–129
						3	14.0	23.4	328	
Skin	1	10.5	14.5	150	72–139	3	10.5	22.4	240	73–136
Kidneys	1	0.82	269	220	72–138	2	0.89	204	180	70–143
Lungs	—	—	—	—	—	2	0.40	122	49	69–145
Spleen	—	—	—	—	—	2	0.19	99.8	19	66–152
Brain	—	—	—	—	—	3	1.44	6.9	10	68–147
Adrenals	—	—	—	—	—	2	0.032	104	3	48–208
Total (excluding liver and intestinal tissue)	—	—	—	620	—	—	—	—	890	—

* Arithmetic means of values for Expts. 2 and 3.

Table 3. Comparison of results obtained from individual organs and from 'carcass' of rats given 1 mg. ergocalciferol (40 000 i.u.)

	1st day		2nd day	
		95% fiducial limits (%)		95% fiducial limits (%)
Total i.u. from individual organs (Table 2)	620	82–122	890	85–117
Total i.u. in 'carcass' (Cruickshank <i>et al.</i> 1953)	690	67–150	630	75–133

Table 4. *Summary of vitamin D assays for Expts. 1-3*

Expt.	Common slope ± s.e.	Residual mean square (with degrees of freedom)	Variance ratios and significance	
			Between slopes	Departure from linearity
1	3.79 ± 0.39	0.6374 (59)	1.70 (0.10 < P < 0.20)	< 1 (P > 0.20)
2	4.23 ± 0.42	0.9335 (74)	1.55 (0.10 < P < 0.20)	< 1 (P > 0.20)
3	3.85 ± 0.35	0.5263 (58)	< 1 (P > 0.20)	2.30 (0.05 < P < 0.10)

The estimate of the vitamin content in the muscle on the second day (for which two replicate groups were available) was obtained by calculating the arithmetic mean of the two separate estimates (Table 2). The fiducial limits for this pooled estimate and for the totals shown in Table 3 were obtained by applying the formula given at the end of Part II of Cruickshank *et al.* (1953).

The significance tests for differences in vitamin content between different tissues, or for the same tissue on different days, are performed by obtaining the difference between the logarithms of the two values, and calculating its standard error in terms of the large sample standard errors of the two log-potencies concerned. As was observed in Part II of Cruickshank *et al.* (1953), these fiducial limits and significance tests take into account only the assay variation. Thus a significant difference between the contents of a particular tissue on the first and second days may reflect merely the biological variation between the two groups of rats used for the two experiments, and it would not necessarily follow that a single group of rats would show a difference in the same direction.

DISCUSSION

We have previously shown (Cruickshank *et al.* 1953) that the amount present in the liver of rachitic rats dosed with ergocalciferol represents about 60 % of the total vitamin in the body on the first day after dosing and about 40 % on the second day. This indicates that the liver is one of the main sites of the absorbed vitamin, at any rate during the short period investigated. This finding is at variance with that of Morgan & Shimotori (1943), who concluded that no one organ was specifically concerned with storage of vitamin D. Despite the low concentration in muscle and skin, these tissues contribute on the first day about 20 %, and on the second day about 40 % of the vitamin content of the entire body. Whether this high content in fact constitutes a store as suggested, at least for skin, by Vollmer (1939) is at present uncertain. The relatively high amount of vitamin contained in the kidneys is of some interest. In contrast to the liver content, which falls by about 1000 i.u. from the first to the second day, the content of the kidney does not decrease significantly. In consequence the concentration on the second day is somewhat

greater than that of the liver. Since no vitamin D appears in urine (Cruickshank *et al.* 1953) it is likely that the kidneys act as a barrier and hence accumulate the vitamin. It is at present a matter of speculation whether this accumulation is of any metabolic significance. It may be associated with the mineral metabolism of the kidneys, or more specifically with the re-absorption of phosphorus by the kidney tubules (Harrison & Harrison, 1941). It may be noted that the concentration of the vitamin in the kidneys is considerably greater than the maximal concentration found in the intestinal tissue (Table 2) which also is concerned with the absorption of minerals. It has long been known that this process is influenced by vitamin D (Harris, 1932), but its exact mode of action is still unknown (Nicolaysen, Eeg-Larsen & Malm, 1953). If vitamin D acts *in situ* its presence in high amounts in the kidneys may be of significance.

Lungs and spleen had a relatively high concentration, as also had adrenals, in which, because of their active sterol metabolism, it may be of particular interest. Brain contained only small amounts, a finding not in accord with that of Houet (1946) who, however, used a colorimetric method of estimation.

The results given in Table 3 provide no evidence that bones and other tissues not examined in the present experiments contained any appreciable amount of the vitamin. Had they done so, the value obtained by summation of the vitamin content of the individual tissues examined in the present experiments would have been less than that obtained for the 'carcass'. In fact, however, the total content of the tissues, other than liver and intestines, obtained on the second day (890 i.u./rat), was significantly higher than the earlier estimate, 630 i.u./rat, for the whole carcass minus liver and intestines. This difference must be ascribed to biological variation between the two groups of animals used in these two series of experiments.

SUMMARY

1. The distribution of vitamin D has been studied in certain tissues of the rat on the first and second days after the oral administration of 1 mg. ergocalciferol (40 000 i.u.).

2. Muscle and skin together contained about 20 % of the body vitamin on the first day and about

40% on the second day but the concentration was low, the estimate lying between 22 and 34 i.u./g.

3. The total vitamin D in the kidneys on the first and second days was estimated to be about 220 and 180 i.u., respectively. The corresponding concentrations were 270 and 200 i.u./g., which were of the same order as that found in the liver in previous experiments. It is suggested that the kidneys accumulate the vitamin by acting as a barrier to its elimination in the urine.

4. In lungs, spleen and adrenals, which contributed little to the total vitamin D content of the body, the concentration was of the order of 100 i.u./g.

5. From the results of the present experiment and those already published, there is no evidence that organs not examined (bones, etc.) contain any appreciable amounts of the vitamin.

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Quantitative Estimation of Glucose by Paper Partition Chromatography

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In the course of recent work it became desirable to develop a simple method for the quantitative estimation of reducing carbohydrates on paper chromatograms, which would be more accurate than that of direct photometry (Gustafson, Sundman & Lindh, 1951) or spot area elution (McFarren, Brand & Rutowsky, 1951), but less time-consuming than elution followed by micro-estimation (Flood, Hirst & Jones, 1948).

It was found that the coloured product formed by heating aniline hydrogen phthalate with glucose on paper could be quantitatively eluted and its concentration determined colorimetrically. Blass, Macheboef & Numez (1950) attempted elution with various organic solvents, but found it incomplete. The technique described below was however found to give reproducible results.

EXPERIMENTAL

Anhydrous glucose was dried to constant weight at 60° *in vacuo*. Quantities equivalent to 50, 100, 150 and 200 μ g. were applied to Whatman no. 4 paper using an 'Agl'a' micrometer syringe. The spots were air-dried and the chromatograms developed 18 hr. by the ascending technique in the following solvent system: *n*-propanol-ethyl acetate-water (7:1:2, v/v) (Baar & Bull, 1953). The chromatograms were then air-dried and drawn through a solution consisting of 85% (v/v) aqueous isopropanol (400 ml.), phthalic acid (6.64 g.) and aniline (3 ml.). Hopkin & Williams 'AnalaR' aniline was redistilled before use. The reagent was kept in a dark bottle

in the refrigerator. Excess of the solution was removed from the papers by means of a rubber roller. The air-dried sheets were placed on the bottom shelf of an electric oven and heated for 15 min. at $115 \pm 1^\circ$. The stained areas were cut out as rectangles allowing a suitable margin and choosing a size suitable to all concentrations. One unstained area of similar dimensions was used to provide a solvent blank. Each of these areas was extracted with glacial acetic acid (4 ml.) at room temperature for 24 hr. The typical absorption curve was established on a Unicam Spectrophotometer (SP. 600) and is shown in Fig. 1. By choosing a wavelength of 480 $m\mu$., a calibration graph was established. This

Fig. 1. Typical absorption curve of coloured product of reaction between glucose and aniline hydrogen phthalate. Colour developed on paper chromatogram and extracted with glacial acetic acid.