recently described a dextrorotatory isomer of i-U, and a racemic DU. While no evidence has yet been presented for their occurrence in nature, their existence enforces the need of distinctive terminology.

Certain additional results given in Table I require brief discussion. In Exp. 2, yield from fresh bile was even greater than from crude glucuronide, yet in Exp. 5 addition of bile salts, either to crystalline bilirubin or crude glucuronide was inhibitory rather than enhancing in its effect. In general, better yields were obtained when there was a 12 hour preliminary growth of bacteria in broth, prior to addition of bilirubin. This is seen most clearly in Exp. 5, Table I.

Recent experiments carried out with S. Gilbertsen, to be described separately, indicate that at least in the normal individual there is a similar basis for failure to note significant increase of fecal urobilinogen after intraduodenal administration of free bilirubin. However, in patients with acholic feces, feeding of above described bilirubin glucuronide has thus far failed to result in formation of appreciable amounts of fecal urobilinogen and other factors must be sought to explain this discrepancy. These studies are being extended.

Summary and conclusions. 1. A method of preparing a relatively stable concentrated adsorbate of conjugated bilirubin (glucuronide) from bile, is described. This has been used in a series of experiments in broth cultures of fecal bacteria. 2. A significant and often much greater increase of urobilinogen formation was observed after addition of the conjugated as contrasted with free bilirubin. 3. The urobilinogen group formed in the bacterial cultures consisted of varying proportions of d-Dehydrourobilin, i-Urobilin, and l-Stercobilin. Under the same conditions the fecal flora readily reduced d-DU to i-U, and i-U to l-S.

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1. Watson, C. J., Graham, A., Ziegler, N., Lowry, P. T., *Trans. A. Am. Phys.*, 1954, v67, 242.

2. a. Schmid, R., *Science,* 1956, v124, 76; b. Billing, B., Lathe, G. H., *Biochem. J.,* 1956, v63, part 1, 6 pp; c. Billing, B., Cole, P. G., Lathe, G. H., *ibid.,* 1957, v65, 774; d. Talafant, E., *Nature,* 1956, v178, 312.

3. Dische, Z., *J. Biol. Chem.,* 1947, v167, 189.

4. Watson, C. J., *ibid.,* 1953, v200, 691.

5. ———, *Ann. Int. Med.,* 1957, v47, 611.

6. Lowry, P. T., Cardinal, R., Collins, S., Watson, C. J., *J. Biol. Chem.,* 1956, v218, 641.

7. Gray, C. H., *Nature,* 1957, v180, 336.

8. Siedel, W., in *Path., Diag. u. Ther. d. Leberkrankh. IVtes Freiburger Symp.,* 1957, p209, Springer Verlag. Berlin.

9. Gray, C. H., *Nature,* 1958, v181, 483.

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Effect of Route of Injection on Distribution and Excretion of Cortisol-4-C^{14} in the Rat. (24159)

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Distribution and metabolism of cortisol-4-C^{14} in rats has been traced after administration by intravenous, intramuscular, subcutaneous, intragastric, and sublingual routes(1,2, 3). Only minor differences in total amount excreted have been found over periods of 1 to 5 days. A previous report(4) described the fate of cortisol-4-C^{14} in the rat during the first hour after intracardiac injection. In normal animals essentially all of the injected radioactivity was recovered in the GI tract and excreta after 60 min. Extension of these studies in which the hormone was injected intravenously, showed a striking difference from previous results, a difference in distribution as well as a slower rate of excretion. This observation was elaborated since it seemed to have application in the study of the role of

TABLE I. Distribution of Radioactivity, in % of Total Counts Injected, after External Jugular Vein Injection of Cortisol-4-C^{14} into Normal Rats.

Time after inj.	5 min.			60 min.		
Wt, g	271	261	263	324	333	327
Dose, μg	12.2	12.2	12.2	13.9	13.9	13.9
, cpm	48,700	48,700	48,700	55,700	55,700	55,700
GI tract & excreta	11.8	11.3	9.2	58.9	56.6	59.4
Muscle	43.7	43.7	38.0	23.6	12.8	21.4
Liver	20.5	23.0	24.4	10.9	13.6	11.1
Skin	16.9	13.1	17.2	9.8	11.1	8.9
Plasma	7.1	5.5	6.9	1.0	2.2	1.1
Kidneys	3.4	3.3	4.2	1.3	1.0	1.1
Head	5.8	5.2	5.7	2.7	2.0	2.9
All other organs*				2.8	2.9	2.9
Total	109.2	105.1	105.6	110.0	102.2	107.9

* Lungs, spleen, heart, thymus, thyroid, pancreas, adrenals, genital organs, and fat.

adrenal cortical hormones in normal and abnormal physiology. First, radioactivity in the principal organs of intact rats was determined 5 and 60 min. after injection of tracer doses of cortisol-4-C^{14} *via* the external jugular and tail veins, and the results were compared with those obtained after intracardiac injection. Second, the study was repeated in adrenalectomized rats 60 min. after external jugular vein injection of cortisol-4-C^{14} alone and cortisol-4-C^{14} mixed with corticosterone.

Materials and methods. The data for intercardiac injection were taken from a previous report(4). For the intravenous injections, 260 to 330 g male Sprague-Dawley rats were used. In the previous studies the rats were fasted overnight prior to intracardiac injection. Since an intact animal fasted overnight was found to have 52.2% of the injected radioactivity in the GI tract and excreta 60 min. after jugular vein injection of 37,900 cpm of cortisol-4-C^{14} (compared to 58.9%, 56.6% and 59.4% in unfasted animals), unfasted animals were used in the studies of intravenous injection. Specific activity of the cortisol-4-C^{14}* was 4.08 μc per mg, 1.0 μg corresponding to about 4,000 counts per min. (cpm) as determined by our counting technic (4,5). The radiochemical purity had been found to be higher than 94%(6). The adrenalectomies were done 8 days before injection by means of a single mid-abdominal incision without aseptic technic. and the animals were maintained on Purina Lab Chow, saline and water. The right external jugular vein was exposed with a ¾ inch incision. A total of 8.5 to 15 μg of cortisol-4-C^{14} in 0.7 to 1.0 ml of 10% propylene glycol in saline was injected over 20 to 25 sec. into the tail or external jugular vein under light ether anesthesia using a one ml tuberculin syringe and #27 needle. In the second group of adrenalectomized animals the injected solution contained 194 μg of corticosterone-C^{12}† in addition to the cortisol-4-C^{14}. Five or 60 min. after injection the animals were sacrificed by exsanguination from the heart. The muscle, liver, skin, kidneys, and head were handled separately; excreta and bladder were combined with the GI tract; all remaining organs and fat were combined and processed as a group. All tissues were dried and dissolved in formamide; the samples were plated on aluminum planchets with lens paper, counted in a Tracerlab Model SC-50 windowless gas flow counter and the activities corrected as previously described(4,5). The plasma was plated directly. All plasma values refer to a total plasma volume calculated from the weight of the animal(7).

Results. In the 5 min. groups (Table I-III) 10% of radioactivity was in the GI tract and excreta. In the intracardiac group the head (after removal of the brain) was processed with the muscles. In the intravenous groups, however, the intact head with the

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† The authors are indebted to The Upjohn Co., Kalamazoo, Mich. for supplying this material.

TABLE II. Distribution of Radioactivity, in % of Total Counts Injected, after Tail Vein Injection of Cortisol-4-C^{14} into Normal Rats.

Time after inj.	5 min.		60 min.					
Wt, g	325	299	255	258	273	314	298	323
Dose, μg	13.0	13.0	12.4	12.4	12.9	13.0	13.0	13.0
" , cpm	51,800	51,800	49,600	49,600	51,800	51,800	51,800	51,800
GI tract & excreta	6.8	9.4	49.6	49.4	43.1	45.2	56.4	53.3
Muscle	42.7	36.5	18.9	17.6	18.6	22.0	18.1	13.2
Liver	20.2	18.0	6.5	6.0	6.3	10.3	5.4	7.2
Skin	16.7	13.5	12.2	14.0	13.3	9.9	9.4	9.3
Plasma	4.0	4.3	1.5	1.3	1.6	1.4	1.3	1.7
Kidneys	3.4	4.3	1.4	1.4	1.2	1.8	1.0	1.1
Head	3.5	4.3	1.9	2.0	2.2	1.8	1.4	1.2
All other organs*	5.0	6.0				3.0	2.0	3.0
Total	102.3	96.3	92.0	91.7	86.3	95.4	95.0	90.0

* See Table I.

TABLE III. Average Distribution of Radioactivity in % of Total Counts of Cortisol-4-C^{14} Injected into Normal Rats by Various Routes.

Time after inj.	5 min.			60 min.		
Route	Intracardiac*	Ext. jugular vein	Tail vein	Intracardiac*	Ext. jugular vein	Tail vein
No. of animals	5	3	2	3	3	6
Wt, g	323	265	312	329	327	287
Dose, μg	14.5	12.2	13.0	14.8	13.9	12.7
" , cpm	58,000	48,700	51,800	59,300	55,700	50,700
GI tract & excreta	11.8	10.8	8.1	98.0	58.3	49.5
Muscle	47.2	41.8	39.6	1.9	19.3	18.1
Liver	25.3	22.6	19.1	4.3	11.9	7.0
Skin	4.4	15.7	15.1		9.9	11.3
Plasma	6.7	6.5	4.2	.7	1.4	1.5
Kidneys	3.7	3.6	3.9	.8	1.1	1.2
Head		5.6	3.9		2.2	1.8
All other organs†	3.7		5.5		2.9	2.7
Total	102.8	106.6	99.3	105.7	107.0	93.1

* Data taken from reference (4). † See Table I.

brain was handled separately. Since the head consisted mainly of attached muscles, it can be seen that total muscle radioactivity content in the 3 groups is about the same. The only striking difference occurred in the skin, (Table III). where radioactivity was higher after both intravenous routes (15.7%, 15.1%) compared to intracardiac injection (4.4%).

Sixty min. after intracardiac injection 98% of the radioactivity‡ had localized in the GI tract and excreta, compared with 58.3% (jugular vein) and 49.5% (tail vein). The portion not excreted 60 min. after intravenous injection was found mainly in the muscle (19 3% and 18.1%) and skin (9.9% and 11.3%).

‡ The comparatively wide range of experimental error(5) should be considered in interpretation of this and all other readioactivity values.

A comparison of our data with that of Ulrich and Long(2) corroborates these findings (Fig. 2). After intracardiac injection we found 6.7%, 4.3% and 0.7% of injected radioactivity in plasma 5, 15 and 60 min. after injection of 14.5 μg of cortisol-4-C^{14}(4). Analysis of these results shows a half-time of disappearance from plasma of 17 min. and a residual after 60 min. of 8.6% of the theoretical zero time activity. After injections of 50 μg of cortisol-4-C^{14} into the jugular vein Ulrich and Long found a longer half-time of disappearance from plasma of 26 min. Extrapolation from their half-life curve gives a residual radioactivity of 20% at 60 min. Although in our jugular vein injection studies we have plasma values only for 5 and 60 min., analysis shows a half-time of 25 min. and a residual radioactivity of 19% at 60 min.

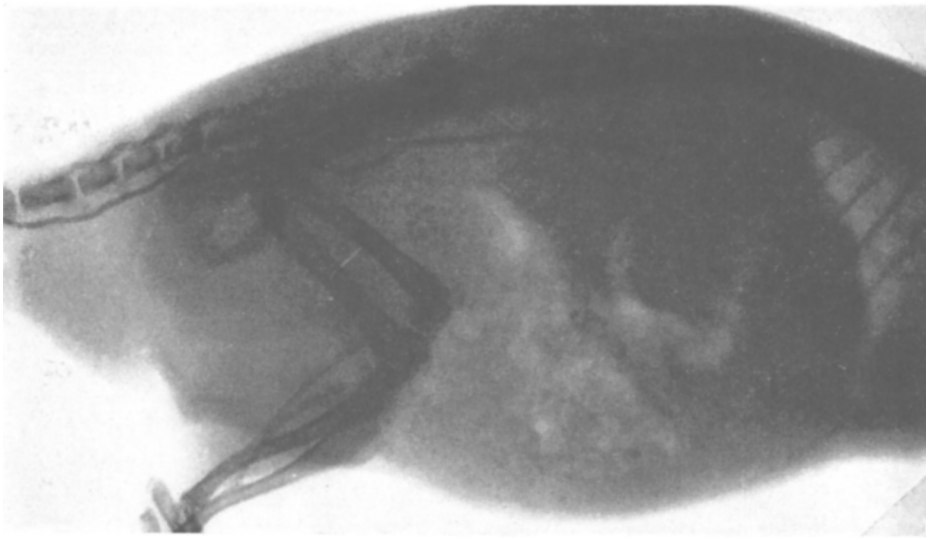

FIG. 1. X-ray during slow (20 to 25 sec.) intrav. inj. of Neo-Iopax.

The high C^{14} content of the skin 5 min. after intravenous injection could have been caused by retrograde flow. X-rays taken while one ml of sodium iodomethanate (50% Neo-Iopax§) was rapidly injected (over 4 to 5 sec) through a #27 needle into the tail vein showed retrograde flow into an abdominal wall vein. However, when the injection was made slowly over 20 to 25 sec (as was done in these studies), all the material went directly into the inferior vena cava (Fig. 1). A similar x-ray study during slow injection of Neo-Iopax into the right external jugular vein showed that the opaque material flowed only into the heart.

Wyngaarden, Peterson and Wolff(1) found 35% and 51% of radioactivity in the bile of 2 cannulated rats one hour after injection of 50 μg of cortisol-4-C^{14}; however, in their studies the steroid was given subcutaneously. Hyde and Williams(3) reported 87% excretion into bile 4 hours after an intravenous dose of 50 μg of cortisol-4-C^{14}. Although both groups have demonstrated an active enterohepatic circulation, this would not appear to be an important factor within 60 min. after injection, in view of the low rate of reabsorption of biliary radiometabolites after intragastric readministration(3).

Corticosterone has been shown to be the predominant corticoid in the rat. Fluctuation of endogenous adrenal output of corticosterone did not seem to be a major influence (Table IV). The amounts excreted during 60 min. in the adrenalectomized animals either with or without added corticosterone were in the same range as in the intact animals.

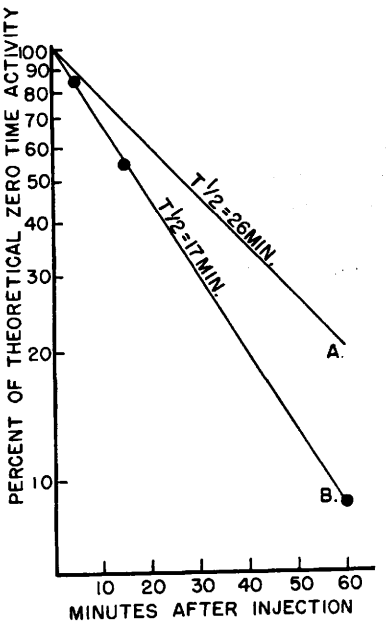

FIG. 2. A = Plasma radioactivity after inj. of 50 μg of cortisol-4-C^{14} into external jugular vein, after Ulrich & Long(2). B = Plasma radioactivity after inj. of 14.5 μg of cortisol-4-C^{14} into heart(4).

§ Schering Corp., Bloomfield, N. J.

TABLE IV. Distribution of Radioactivity, in % of Total Counts Injected, 60 Min. after External Jugular Vein Injection of Cortisol-4-C^{14} (Alone or with Corticosterone) into Adrenalectomized Rats.

Rat No.	1	2	3	Avg	1	2
Wt, g	286	297	297	293	308	280
Cortisol-4-C^{14}, μg	12.0	12.0	12.0	12.0	8.5	8.5
" , cpm	47,700	47,700	47,700	47,700	34,000	34,000
Corticosterone, μg					194	194
GI tract & excreta	39.9	37.8	51.0	42.9	47.4	47.4
Liver	11.7	11.9	8.7	10.8	8.1	10.6
Kidneys	3.2	2.3	2.4	2.6	1.6	1.9
Plasma	3.3	3.1	2.7	3.0	1.6	1.5

The liver is the main site of metabolism of cortisol in mammals, and in the rat it is also the main excretory organ. Englert and coworkers(8) have clearly shown that rate of disappearance of infused cortisol from the plasma of dogs is proportional to hepatic blood flow. In our experiments more rapid excretion of radioactivity after intracardiac injection was probably due to an acceleration of hepatic blood flow during the 1 hr after the puncture. A simultaneous decrease in blood flow through the skin could explain the low initial content in that organ. Such circulatory changes could well be produced by neural or endocrine reactions to the injection puncture. Cardiac puncture is a traumatic procedure undoubtedly causing prompt vasomotor changes and leaving injury to an extremely sensitive tissue well beyond the experimental period with unknown changes in the rates and patterns of arterial blood distribution. Manipulation of the jugular vein as it is dissected free as well as the venipuncture have frequently caused marked local venospasm. The tail vein injection, on the other hand, would appear to be the most innocuous route and should cause the least changes in circulatory dynamics. Patterns of distribution and slower rates of excretion after the intravenous injection would, therefore, more closely approximate the fate of endogenous hormone.

In view of these findings, previous studies on clearance of injected steroids should be interpreted in the light of possible hemodynamic changes caused by experimental conditions or manipulations and in such studies these changes should be controlled or measured, especially when using small animals.

Summary. Rate of excretion of radioactivity by rats was more rapid after intracardiac injection of cortisol-4-C^{14} than after intravenous injection. In addition, after the latter route significantly larger quantities of radioactive material entered the skin immediately following injection. The most likely explanation for this difference is considered to be hemodynamic changes caused by heart puncture. The tail vein is the most suitable site of intravascular injection for studies of the fate of adrenal steroids in normal animals under basal conditions.

1. Wyngaarden, J. B., Peterson, R. E., Wolff, A. R., *J. Biol. Chem.*, 1955, v212, 963.

2. Ulrich, F., Long, C. N. H., *Endocrinol.*, 1957, v59, 70.

3. Hyde, P. M., Williams, R. H., *J. Biol. Chem.*, 1957, v227, 1063.

4. Firschein, H. E., DeVenuto, F., Fitch, W. M., Pearce, E. M., Westphal, U., *Endocrinol.*, 1957, v60, 347.

5. Pearce, E. M., DeVenuto, F., Fitch, M., Firschein, H. E., Westphal, U., *Anal. Chem.*, 1956, v28, 1762.

6. Westphal, U., Firschein, H. E., Pearce, E. M., *Am. J. Physiol.*, 1956, v185, 54.

7. Berlin, N. I., Huff, R. L., Van Dyke, D. C., Hennessy, T. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 176.

8. Englert, E., Jr., Nelson, R. M., Brown, H., Nielsen, T. W., Chou, S. N. Cited by L. T. Samuels, Ciba Foundation Colloquia on Endocrinology, 1957, v11, 222. Little, Brown and Co., Boston, 1957

Received May 6, 1958. P.S.E.B.M., 1958, v98.