present in the supernatant medium. These findings suggest that human pituitary cells grown in tissue culture do not retain their functional properties for growth hormone production under the described culture conditions.

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1. Knobil, E., Greep, R. O., *Rec. Prog. Horm. Res.*, 1959, v15, 1.

2. Li, C. H., *Postgraduate Med.*, 1961, v29, 13.

3. Hinz, R. W., Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 19.

4. Li, C. H., Moudgal, N. R., Papkoff, H., *J. Biol. Chem.*, 1960, v235, 1038.

5. Marshall, J. D., Eveland, W. C., Smith, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 898.

6. Coons, A. H., Leduc, E. G., Connolly, J. M., *J. Exp. Med.*, 1955, v102, 49.

7. Vazquez, J. J., Dixon, F. J., *ibid.*, 1956, v104, 727.

8. Thompson, J. W., Vincent, M. N., Jensen, F. C., Price, R. T., Schapiro, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 403.

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Acid Phosphatase and β-Glucuronidase Activities of Thymus and Spleen of Rats after Whole-Body X-Irradiation.* (27209)

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On the basis of differential centrifugation, de Duve and co-workers(1,2) proposed the name "lysosome" for a special group of intracellular particles which show a sedimentation distribution between the mitochondrial and the microsomal fractions; this group contains a number of easily soluble hydrolases (*i.e.*, acid phosphatase, β-glucuronidase, acid ribonuclease, deoxyribonuclease II, cathepsin, etc.) having in common an acid pH optimum (3).

To study the effect of x-irradiation on this particular group of particles, we have taken the thymus and the spleen of rats as our study object, since lymphoid tissue is far more radiosensitive than other tissues in which lysosomes have been studied. Among the lysosomal group of enzymes, so far we have tested only acid phosphatase and β-glucuronidase.

Methods. X-irradiation procedure. Two sets of experiments were carried out with female Holtzmann rats. A total of 32 animals, 45 to 50 days old and weighing between 130 and 150 g, were studied for enzyme activities in Experiment 1; 24 of them were given a dose of 200 r, and the remaining 8 were used as controls. In Experiment 2, 48 animals were studied; 36 of them were given a single dose of 1000 r, and the remaining 12 were used as controls. The average dose rate was 50 r/min in Exp. 1, and 190 r/min in Exp. 2, delivered by a 250 kv machine with 0.5 mm Cu and 3.0 mm bakelite filters; the target distance was 29 cm, and the rats were rotated during exposure.

Since we found that the control values (especially for acid phosphatase) varied between different shipments of rats, we have used a single shipment of rats for each set of experiments. The acid phosphatase and β-glucuronidase specific activities were found to be constant among individual rats in the same shipment. For the thymus (rats from 42 to 130 days of age), values for acid phosphatase range from 0.26 to 0.28 μg P/mg N/min and from 5.8 to 6.3 μM phenolphthalein/g N/min for β-glucuronidase. As for the spleen (rats from 45 to 70 days of age), values range from 0.39 to 0.42 μg P/mg N/min and 1.52 to 1.70 μM phenolphthalein/g N/min respectively.

Tissue preparations and enzymes assays. The animals were killed by decapitation, and thymus and spleen were removed and chilled at once in 0.25 M sucrose at 0°C; the tissue was then cut into a few small pieces and ho-

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TABLE I. Effect of Whole-Body X-Irradiation of Thymus of Rats at a Single Dose of 200 r.

Time after irrad. (hr)	Wet wt (mg)	Total nitrogen (mg)	Acid phosphatase E.S.A.	Acid phosphatase E.T.A.	Acid phosphatase F.A.	β-glucuronidase E.S.A.	β-glucuronidase E.T.A.	β-glucuronidase F.A.
Control	423	16.3	.24	3.91	49	5.6	91	47
5	353	16.4	.21	3.44	55	4.5	74	62
24	200	5.9	.66	3.87	68	14.6	86	59
48	116	4.0	.64	2.59	48	14.1	57	48

Each value represents avg of 8 animals.

E.S.A. = Enzyme specific activity, μg P/mg N/min. for acid phosphatase, and μmoles phenolphthalein/g N/min. for β-glucuronidase.

E.T.A. = Enzyme total activity, product of total nitrogen and E.S.A.

F.A. = Free activity, expressed in % of total activity.

mogenized in a cellulose nitrate test tube fitted with a glass pestle. The large quantity of connective tissue in these organs gave some difficulty in pipetting and hence in satisfactory reproducibility of the enzyme assays. This difficulty was overcome by passing the tissue homogenate through a piece of gauze which retained the connective tissue.

Free activities were estimated in presence of a substrate mixture containing 0.25 M sucrose, with an incubation time of 10 minutes. Total activities were estimated in the presence of 0.1% Igepal-630, which was found to have the same effect as Triton X-100 on releasing the enzymes completely from the particulate membrane of the lysosomes. The enzyme assays were essentially the same as described by Gianetto and de Duve(4).

Results. Thymus. Exp. 1 (200 r): The thymus wet weight showed a decrease of 16.4% as early as 5 hours after irradiation, but total nitrogen was almost unchanged, so that the decrease in wet weight was probably due simply to a loss of water content of the organ. Twenty-four and 48 hours after irradiation the decrease in wet weight was parallel to the loss of total nitrogen content. As far as enzyme specific activities are concerned, both acid phosphatase and β-glucuronidase showed a slight decrease after 5 hours, but an increase of almost 3-fold over control value after 24 hours for both enzymes (Table I). After 48 hours, enzyme specific activities showed a tendency to decrease. *Exp. 2 (1000 r)*: The decrease in wet weight and in total nitrogen was similar to that of Exp. 1, but specific activities showed a greater increase after 48 hours (Table II).

Spleen. Exp. 1 (200 r): For both the enzymes the response of the spleen was essentially similar to that of the thymus to a dose of 200 r, (Table III), except that the increase of enzyme specific activities after 48 hours was greater than that after 24 hours. *Exp. 2 (1000 r)*: Under these conditions, acid phosphatase seemed to show a greater increase as early as 2 hours, but continued to increase slowly 24 and 48 hourse after irradiation (Table IV). β-glucuronidase activity showed a slight increase after 2 hours, but increased 2-fold at 24 hours, and almost 3-fold at 48 hours.

Discussion. Eichel and Roth(5) and Feinstein(6) among others, have pointed out that it is extremely difficult to intrepret tissue enzyme changes after whole-body x-irradiation particularly in the lymphoid organs such as the spleen and thymus in which there is a considerable loss of weight and nitrogen after such treatment.

TABLE II. Effect of Whole-Body X-Irradiation on Thymus of Rats at a Single Dose of 1000 r.

Time after irrad. (hr)	Wet wt (mg)	Total nitrogen (mg)	Acid phosphatase E.S.A.	Acid phosphatase E.T.A.	Acid phosphatase F.A.	β-glucuronidase E.S.A.	β-glucuronidase E.T.A.	β-glucuronidase F.A.
Control	433	16.2	.15	2.42	66	5.6	91	48
2	379	16.4	.19	3.12	64	4.9	81	51
24	211	9.5	.20	1.90	74	8.4	80	56
48	54	2.6	.64	1.68	53	27.7	73	58

Each value represents avg of 12 animals.

See Table I for abbreviations and units.

TABLE III. Effect of Whole-Body X-Irradiation on Spleen of Rats at a Single Dose of 200 r.

Time after irrad. (hr)	Wet wt (mg)	Total nitrogen (mg)	Acid phosphatase			β-glucuronidase		
			E.S.A.	E.T.A.	F.A.	E.S.A.	E.T.A.	F.A.
Control	510	28.7	.38	10.90	53	1.5	43	60
5	410	32.5	.31	10.10	56	1.4	46	65
24	264	16.2	.56	9.10	51	2.2	36	57
48	225	13.1	.69	9.03	52	2.4	31	62

Each value represents avg of 8 animals.
See Table I for abbreviations and units.

TABLE IV. Effect of Whole-Body X-Irradiation on Spleen of Rats at a Single Dose of 1000 r.

Time after irrad. (hr)	Spleen wet wt (mg)	Total nitrogen (mg)	Acid phosphatase			β-glucuronidase		
			E.S.A.	E.T.A.	F.A.	E.S.A.	E.T.A.	F.A.
Control	633	43.1	.20	8.62	52	1.3	56	45
2	560	33.0	.36	11.90	52	1.4	46	54
24	286	16.2	.41	6.65	48	2.5	41	55
48	157	10.4	.52	5.38	61	3.3	34	61

Each value represents avg of 12 animals.
See Table I for abbreviations and units.

From the data shown in Table I to IV, the enzyme changes found after 24 and 48 hours seem to indicate quite clearly that one can interpret these changes as a result of a selective nitrogen loss since total organ enzyme activities (for both acid phosphatase and β-glucuronidase) were unchanged or slightly decreased even though specific activities increased up to 3-fold.

However, the data obtained 2 hours after 1000 r for acid phosphatase showed a moderate increase in enzyme specific activity and a 30 to 40% increase in total organ enzyme activity, both in thymus and in spleen. An increase as early as within 2 hours seems to eliminate the possibility of either a synthesis of the enzyme or a selective nitrogen loss, since the cell population of the organ was not materially changed 2 hours after irradiation. There is no indication of either the release of any activator or removal of an inhibitor after x-irradiation, as far as acid phosphatase is concerned (unpublished work).

The measurement of the free activity (tested in the medium containing 0.25 M sucrose in which the lysosomal particles remained intact) did not show a significant difference even after a dose of 1000 r; this observation supports the results obtained by Bacq(7), that a dose of more than 10,000 r was required before a leakage of hydrolytic enzymes from isolated lysosomes can be detected. He suggested that the possibility remained that lysosomes are more sensitive within the cell. This possibility still cannot be ruled out on the basis of our data, since the largest dose we have given was only 1000 r; the breakdown of the lysosomes *in vivo* probably requires a dose higher than 1000 r.

Thus the mechanism of the early increase of acid phosphatase (specific activity as well as total organ activity) remained obscure. It is still more difficult to determine its relationship to the lysosome particles in the cells even though from the physiological point of view the necessity of an increase of lysosomal enzymes whenever there is increased autolysis is obvious(8). Another puzzling point is that β-glucuronidase did not follow the same pattern of increase 2 hours after 1000 r; this enzyme, in contrast to acid phosphatase, showed a slight decrease both in specific and total organ activity. More work remains to be done before we can answer these unexplained points.

Summary. Acid phosphatase and β-glucuronidase activities of thymus and spleen from rats after whole-body x-irradiation were studied. The increases found in specific activities 24 and 48 hours after a single dose of 200 r and 1000 r could be interpreted on the basis of a selective nitrogen retention in the tissues

after radiation, but the increase of acid phosphatase activity in the thymus and spleen 2 hours after 1000 r remained unexplained. The percentage of free activities of these 2 enzymes did not seem to be affected by radiation.

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1. Appelmans, F., Wattiaux, R., de Duve, C., *Biochem. J.*, 1955, v59, 438.
2. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., Appelmans, F., *ibid.*, 1955, v60, 604.
3. de Duve, C., in: *Subcellular Particles.* Am. Physiolog. Soc. Washington, D. C., 1959, p. 128.
4. Gianetto, R., de Duve, C., *Biochem. J.*, 1955, v59, 433.
5. Eichel, H. J., Roth, J. S., *Radiation Res.*, 1960, v12, 258.
6. Feinstein, R., *ibid.*, 1956, v4, 217.
7. Bacq, Z. M., Alexander, P., in *Fundamentals of Radiobiology,* Pergamon Press, New York, 1960, p. 274.
8. de Duve, C., *Exp. Cell Res.*, Suppl. 7, 1959, 169.

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Evidence for a Direct Effect of Angiotensin-II on Adrenal Cortex of the Dog.*† (27210)

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Angiotensin-II stimulates aldosterone secretion in normal humans(1,2) and nephrectomized hypophysectomized dogs(3,4). This could be due to a direct action on the adrenal, or it could be an indirect effect. It has been claimed that some polypeptide hormones and other substances release ACTH from binding sites in the body(5,6) and angiotensin-II might have this action. Its hemodynamic effects might also cause liberation of an aldosterone stimulating substance. To investigate the possibility of an indirect effect, we have compared the changes in adrenocortical secretion produced by infusing angiotensin-II into the arterial supply of the adrenal gland with the changes produced by its infusion into a peripheral vein in nephrectomized hypophysectomized dogs.

Methods. Six male mongrel dogs weighing 9.4 to 16.2 kg were used. The right kidney and the right adrenal were removed and the right lumbo-adrenal artery and the 2 pairs of lumbar arteries leaving the aorta at the level of the adrenals and the kidney tied. Six to 36 days later, each dog was anesthetized with pentobarbital and the left lumbo-adrenal vein cannulated. The left kidney was removed and the aorta and vena cava tied just below the renal vessels (Fig. 1). The left lumbo-adrenal artery was tied 1 cm lateral to the left adrenal. All branches to the diaphragm were ligated. A PE-50 polyethylene cannula was then inserted into the lumbo-adrenal artery, adjusted so that its tip was just at the aortic orifice of the lumbo-adrenal artery, and tied in place. The left carotid artery was cannulated for continuous monitoring of blood pressure with a Statham strain gauge and Grass Model 4 polygraph. A PE-50 cannula for infusing angiotensin-II and a larger cannula for administering transfusions were inserted into the left jugular vein. The dog was then hypophysectomized via the transbuccal route. Two control adrenal venous blood specimens were collected 60 and 80 minutes after hypophysectomy. Synthetic angiotensin-II in saline was then infused into the jugular vein (in 2 dogs) or the lumbo-adrenal artery (in 4 dogs) for 14 minutes.

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