served represents the humoral factor that has been implicated in recovery of mammals from the damaging effects of ionizing radiations.

Summary. 1. A prolongation of the lag phase in irradiated *E. coli* is described. The modifying effects of temperature during growth, storage of irradiated organisms, and the addition of mouse blood, inactivated rabbit serum, 0.5% cysteine solution, the filtrate of actively growing *E. coli* cultures, fresh spleen, liver and kidney mash, or growing non-irradiated *E. coli* cultures, have been studied. 2. Evidence that sublethal effects of X irradiation in bacteria differ from lethal effects is discussed.

1. Robinson, R. F., Phillips, M. D., and Nagelsen, M. G., A bibliography on the effects of X ray on bacteria, Battelle Memorial Inst., Aug. 20, 1951.

2. Stapleton, G. E., Hollaender, A., and Martin, F. L., *J. Cell. Comp. Physiol.*, 1952, v39, sup. 1.

3. Beutler, E., and Robson, M. J., unpublished data.

4. Stapleton, G. E., Billen, D., and Hollaender, A., *J. Cell. Comp. Physiol.*, 1953, v41, 345.

5. Hollaender, A., and Stapleton, G. E., *Physiol. Rev.*, 1953, v33, 77.

6. Giese, A. C., *Quart. Rev. Biol.*, 1947, v22, 253.

7. Lea, D. E., *Actions of Radiation on Living Cells*, Cambridge, England, University Press, 1946.

8. Rubin, B. A., *The relation of nucleic acid metabolism to the genetic effect of radiation in bacteria*, NSA, 1951, v5, 4953, AECU-1436.

9. Lea, D. E., Haines, R. B., and Coulson, C. A., *Proc. Roy. Soc.*, London, 1937, vB123, 1.

10. Jacobson, L. O., *Cancer Research*, 1952, v5, 315.

11. Hollaender, A., Stapleton, G. E., Burnett, W. T., Jr., *The Modification of X ray Sensitivity by Chemicals*, "Isotopes in Biochemistry, a Ciba Foundation Conference," Churchill Ltd., London, 1951, 96.

Received April 2, 1954. P.S.E.B.M., 1954, v85.

Ribonuclease Activity of the Mouse Pancreas. (20993)

M. RABINOVITCH.* (Introduced by I. Gersh.)

From the Departments of Anatomy and Medicine, University of Chicago, Chicago, Ill.

Although the activities of several enzymes have been studied in the pancreas after fasting(1-3) and after the administration of cholinergic drugs to normal animals(3-6), no data bearing on pancreatic ribonuclease could be found. Information on this enzyme is of particular interest because of the occurrence of large amounts of ribonucleic acid(7) and because the pancreas is a rich source of crystalline ribonuclease. The evidence presented in this paper indicates that ribonuclease activity of pancreatic homogenates is reduced by fasting and by the administration of pilocarpine.†

Materials and methods. Adult white mice fed Purina dog chow were used. Males were studied, with the exception of those in Exp. I, Table I. In the fasting experiments the mice received only water for the times indicated. For the pilocarpine experiments, the fasted or unfasted mice regardless of body weight were injected intravenously with 2 doses of 0.6 mg each of pilocarpine hydrochloride (total volume 0.6 ml) at 30 minute intervals. Controls were similarly injected with distilled water. Intense salivation, lacrimation and diarrheic stools followed the injections. The mice were killed by a blow on the head, and weighed. The pancreas was removed, and weighed on a torsion balance. 0.1% homogenates were prepared with chilled distilled water, and the homogenates stored at −10°C until the determinations of enzyme activity were performed, within the next 18 hours. Control and pilocarpine-treated mice from each experiment were injected and killed alternately, and determinations of enzyme activity were performed simultaneously. Comparisons are to be made only within the individual experi-

* Fellow of the Rockefeller Foundation. Permanent address: Dept. of Histology and Embryology, Faculty of Medicine, University of São Paulo, Brazil.

† After completion of this paper a preliminary communication appeared(8) which claims that a reduction of the ribonuclease activity of the mouse takes place after the injection of carbamyl choline.

TABLE I. Influence of Fasting on the Ribonuclease Activity of the Pancreas of the Mouse.

Exp.	Treatment	No. of animals	Avg wt in g	Ribonuclease activity*	Stand. dev.
I †	a) Control	13	26.8	.264	.038
	b) 12 hr fast	7	29.1	.256	.045
	c) 24 " "	6	26.8	.194	.015
II‡	a) Control	10	28.8	.208	.042
	b) 30 hr fast	10	22.7	.165	.040

* Increase in opt. dens. at 260 mμ, produced by 13.3 μg fresh tissue.
† F found 8.24, $P < 1\%$; t tests, groups a-b, $P > 0.6$; a-c, $P < 0.001$; b-c, $P < 0.01$.
‡ $t = 2.35$, $P < 0.05 > 0.02$.

ments. *Ribonuclease activity* determinations were made by reading at 260 mμ(9) in a Beckman model DU spectrophotometer the absorption due to soluble nucleotides liberated from yeast nucleic acid by enzymatic activity of the homogenate. The composition of the substrate was the following: 0.5 ml of 0.05 M phosphate buffer pH 7.3; 0.3 ml of a 0.35% solution of commercial yeast ribonucleic acid, purified according to Kunitz(10) dissolved in 0.1 M sodium acetate and dialysed against 0.1 M sodium acetate at 4°C for at least 48 hours before use. To this substrate was added 0.2 ml of homogenate diluted 1:15, and the mixture was incubated for 20 minutes at 37°. Enzyme activity was interrupted, and undigested ribonucleic acid precipitated by the addition of 2 ml of 1 N HCl in 80% alcohol(11). After agitation and standing for one hour at 4°C, the tubes were centrifuged for 15 minutes in a refrigerated centrifuge, and aliquots of the supernatants taken for spectrophotometry. Blanks were run by adding the homogenates to the substrate after addition of the acid alcohol. Results are expressed in increase of the optical densities over those of the blanks, when an equivalent of 13.3 micrograms of fresh tissue was used for the determinations. In experiments with crystalline ribonuclease it was found that an optical density increase of 0.200 over the blanks was obtained with 0.005 μg of the enzyme. Previous results(3,12), did not disclose significant wet weight/dry weight ratio modifications in the mouse pancreas after the administration of pilocarpine.

Under these conditions of enzyme determination, it was found that there was a direct proportionality between the amount of pancreatic tissue used and enzyme activity. Activity of the homogenates was not modified by their storage at 4°C for 48 hours. Magnesium sulfate (final concentration 0.06 M) led to 50% inhibition of the activity of the homogenates, which agrees with previous data obtained with the crystalline enzyme(13). No modification in the activity of the homogenates was found when they were prepared with either 0.1% "Versene" solution (pH around 6), 0.1% saponin (pH around 7.0) or 0.15 N H_2SO_4.

Results. Table I shows that 24 and 30 hours, but not 12 hours, of fasting decreased the ribonuclease activity of the pancreas. Table II shows that one hour after the administration of pilocarpine the average ribonuclease activity of the pancreas is decreased significantly. Twelve hours after the administration of pilocarpine (exp. III), the ribonuclease activity of the pancreas has recovered to normal values of control mice.

Discussion. The decrease in ribonuclease activity of the pancreas in fasting mice agrees with the findings of others on protease(1,2), amylase(2,3) and lipase(2). The biochemical assays also agree with the histological descriptions of Okuneff(14), who reported reduction in the number of zymogen granules in the rabbit pancreas after prolonged fasting. The early return to normal levels after only 12 hours following the injection of pilocarpine also corresponds with the time when the secretory granules reach their normal density in the apical pole of the acinar cells. It is interesting also that Marshall(15) was able to localize ribonuclease in the apical region by a specific cytochemical method. For these reasons, it is possible to suggest that at least part of the ribonuclease in the pancreas is excreted in the pancreatic juice. The possi-

TABLE II. Influence of Pilocarpine Administration on the Ribonuclease Activity of the Pancreas of the Mouse. 4 experiments.

Time (hr) after 1st inj.	Treatment	No. of animals	Avg wt in g	Ribonuclease activity*	Stand. dev.	Probabilities (*t* test)
1	U.c.†	10	17.6	.183	.043	$<.01$
	P.	9	16.8	.120	.025	
1	U.c.	13	23.4	.192	.038	$<.001$
	P.	12	23.2	.128	.030	
12	U.c.	10	27.1	.169	.039	$>.7$
	P.	9	26.0	.175	.057	
1	6 hr F.c.	11	24.6	.214	.077	$\gtrsim.05$
	P.	11	25.2	.156	.053	

* Increase in optical density at 260 mμ produced by 13.3 μg fresh tissue.
† U.c. = unfasted controls; P. = pilocarpine; F.c. = fasting controls.

bility of vascular discharge cannot be excluded(16).

Summary. Twenty-four and 30 hours fasting reduces ribonuclease activity of the pancreas of mice. One hour after the administration of pilocarpine a significant drop of enzymic activity takes place, with recovery in less than 12 hours.

The author is indebted to Professors I. Gersh and E. S. G. Barron for helpful suggestions.

1. Heidenhain, R., *Arch. ges. Physiol.*, 1875, v10, 557.
2. Bocchioti, S., *Bol. Soc. Ital. Biol. Sper.*, 1948, v24, 952.
3. Daly, M. M., and Mirsky, A. E., *J. Gen. Physiol.*, 1952, v36, 243.
4. van Weel, P. B., *Z. vergl. Physiol.*, 1939, v27, 311.
5. van Weel, P. B., and Engel, C., *ibid.*, 1938, v26, 67.
6. Hokin, L. E., *Biochem. J.*, 1951, v48, 320.
7. Davidson, J. N., *The Biochemistry of the Nucleic Acids*, Methuen, London, 1950.
8. Dickman, S. R., and Kropf, R., *Fed. Proc.*, 1954, v13, 199.
9. Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, 1952, v198, 155.
10. Kunitz, M., *J. Gen. Physiol.*, 1940, v24, 15.
11. Roth, J. S., and Milstein, S. W., *J. Biol. Chem.*, 1952, v196, 489.
12. Rabinovitch, M., Valeri, V., Rothschild, H. A., Camara, S., Sesso, A., and Junqueira, L. C. U., *ibid.*, 1952, v198, 815.
13. Lamanna, C., and Mallette, M. F., *Arch. Biochem.*, 1949, v24, 451.
14. Okuneff, N., *Arch. mikr. Anat.*, 1923, v97, 187.
15. Marshall, Jr., J. M., *Exp. Cell. Res.*, 1954, v6, 240.
16. Popper, H. L., and Necheles, H., *Gastroenterology*, 1943, v1, 490.

Received April 6, 1954. P.S.E.B.M., 1954, v85.