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Island-Cell Tumors in Irradiated Rats in Parabiosis.* (29075)

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The incidence, function and age and sex distribution of adenomas and carcinomas of the islands of Langerhans were studied in Deac-Slonaker rats. These lesions are very rare in rats, and we have not encountered them in normal control rats of this or other strains. In the course of experiments to determine the effects of a single supralethal dose of radiation on duration of life and incidence of tumors we parabiosed young inbred littermate rats of this strain. The technique, described elsewhere(1), was to irradiate one of the pair some weeks after parabiosis and when equilibrium had been established between the animals. The right-hand rat received a single supralethal dose of 1000 r of 250 KV X-rays. The left-hand rat was shielded. The LD₅₀ for single rats is 750 r. Parabiosis protects the supralethally irradiated animal from death and from the most marked effects of the acute radiation syndrome. Parabiosis in itself provides an abnormal hormonal balance in the animals, which is accentuated by the irradiation of one. We found tumors of the islands of Langerhans rarely in control parabiotic rats, commonly in the irradiated partner. The ani-

mals were allowed to live until dead or moribund.

For this study 715 pairs of parabiont rats were chosen at random, but with life spans of over 200 days after operation or irradiation. One hundred thirteen pairs of these parabiosed animals, approximately equally divided as to sex, were used as controls. Of the irradiated pairs 276 were male to male parabionts, 326 female to female. The findings are set out in Tables I and II. Males are strikingly prone to develop these pancreatic tumors.

Morphology. The tumors of the islands are usually large enough to be seen grossly (0.4 to 1.2 cm). The appearance of the benign tumors was fairly uniform, and they could be separated morphologically into two types.

The first type closely followed the architecture and vascularization of the normal island, notwithstanding the distortion caused by the growth process. The central portion maintains an orderly arrangement with short and narrow cell cords; the periphery is less orderly and occasional "blood lakes" are present. Capsule formation may be incomplete. Some lesions retain alpha cells along with the dominant beta cells. These adenomas perhaps could be classed as "giant islands."

The second type more obviously meets the neoplastic criteria of Borst(2): it shows marked structural variations from the normal

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TABLE I. Tumors of Islands of Langerhans in Parabiont Rats.

	Control partners				Experimental partners							
					Unirradiated parabiont				Irradiated parabiont			
	♂		♀		♂		♀		♂		♀	
	#	%	#	%	#	%	#	%	#	%	#	%
Adenoma	1	1	0	0	2	0.7	0	0	33	12	7	2
Carcinoma	0	0	0	0	0	0	0	0	2	.7	0	0

pattern, the cell cords are much thicker and longer and the capillary bed is reduced. The periphery of the adenoma shows evidence of expansion at the expense of the surrounding parenchyma, as witnessed by the compressed acinar tissue and occasional presence of acini trapped in the expanding insular tissue. Rather thick capsules are characteristic for this type. The presence of "blood lakes" is a constant finding.

The two carcinomas resembled this latter type, but were invasive and showed mitotic activity.

Function. Two adenomas were extracted for insulin by the acid ethanol procedure(3), and the supernatant fluid was assayed for insulin content by the rat adipose tissue technique, where oxidation of labeled C^{14} glucose to $C^{14}O_2$ is the index of activity(4). Each unknown was assayed at 3 different dilutions in triplicate. Both tumors contained between 60 and 80 units per gram wet weight. Normal rat pancreas contains between 1 and 3 units per gram wet weight. Values for rat islet cell adenoma are similar to those reported in some cases of human islet cell tumors(5).

The carbohydrate metabolism of a number of pairs was studied, but the results fail to correlate with the presence of adenomas. Some random pairs of rats showed intermit-

tent glycosuria. This trait did not correlate with subsequent demonstration of insular adenomas. A series of random, usually terminal, blood sugar determinations in different animals ranged from 68 to 214 mg %. Values tend to agree when repeated in the same animal. The low blood sugar value for animals proved later to have adenomas was also 68, the high 214 mg %.

Comment. As a working hypothesis the following conjecture is put forward: radiation damages the irradiated rat's pancreas and possibly pituitary as well; the damaged islands are overburdened by the metabolic needs and diabetes develops.

This diabetic state shows an exaggerated sexual difference, being much more frequent in males. This sex ratio is equal or greater than that which Foglia has studied in the subtotally pancreatectomized rat(6,7). The formation of adenoma is interpreted as a compensatory phenomenon. The frequency of adenoma increases steadily with age (Table II). Occasionally the tumors were multiple. Renal damage from irradiation may produce disturbances that might affect the glycosuria and glycemia. Irradiation of the region of the pancreas has been reported to produce wave-like fluctuation in secretion of exocrine enzymes(8), but no data exist showing influence on insulin secretion. The predilection of island cell tumors for male irradiated rats (Table I), noted also by Rosen(9,10), seems to correlate with the above described greater frequency of diabetes in the male.

TABLE II. Incidence of Adenoma by Age Post-irradiation.

Days post-irradiation	Male parabionts				(%)
200-299	0 out of	64 pairs—	0		
300-399	1 " "	39 " "	2.5		
400-499	6 " "	69 " "	8.6		
500-599	11 " "	62 " "	17.7		
600-699	6 " "	25 " "	24.0		
700-799	9 " "	17 " "	52.9		
	33 " "	276 " "	11.8		

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Specific Detection of Phenylalanine Following Chromatographic Separation from Serum and Urine of Phenylketonuric Patients. (29076)

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Previous studies from our laboratory have shown that a specific reaction for phenylalanine in complex biological mixtures is produced when the ninhydrin-stained chromatograms are subsequently treated with dilute sodium bicarbonate(1). It was also later shown that the same reaction can be used for a quantitative method that permits determination of phenylalanine on paper chromatograms in the presence of other amino acids even under conditions of poor separation and resolution(2). In this communication is shown how this reaction may be applied to serum and urine of phenylketonuric (PKU) patients following paper chromatographic separation of the amino acids. The paper chromatographic procedure used by Berry(3) was also studied.

Procedure. Chromatography. Two chromatographic procedures were applied to whole blood, serum and urine samples from PKU patients, (1) descending one-dimensional paper chromatography and (2) horizontal one-dimensional circular paper chromatography. These samples were compared with pooled lots of normal whole blood, normal serum and normal urine. All PKU urine samples were first morning voids, and blood samples containing EDTA as anticoagulant

were taken shortly afterwards. Volumes of whole blood and serum ranging from 0.1 to 5 ml and urine specimens in 5 ml amounts were treated as follows:

One volume of absolute alcohol was added to blood or serum specimens and mixed well with a vortex mixer. After standing for 15 minutes at 0° to 4°C they were centrifuged at 2000 rpm for 20 minutes. The precipitated proteins were then washed twice with approximately 50% alcohol (v/v); all the supernatants were pooled and then dried over concentrated sulphuric acid *in vacuo*. These were then reconstituted into 50 or 100 μ l of distilled water and appropriate volumes applied to paper in order to detect concentrations above 3 mg % of phenylalanine. Five milliliter aliquots of the urine samples were dried directly over concentrated sulphuric acid *in vacuo* without the alcohol treatment. The dried urine specimens were reconstituted to 0.2 or 0.3 ml with distilled water and suitable microliter volumes spotted on the filter paper.

Descending one-dimensional chromatography was carried out with the redeveloping technique as described previously(4,5,6). In addition, circular one-dimensional horizontal paper chromatography was employed for