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Lesions of the Islands of Langerhans Produced by a Styryl Quinoline Derivative.

F. D. W. LUKENS AND W. B. KENNEDY.

From the George S. Cox Medical Research Institute, University of Pennsylvania.

Dunn, Sheehan and McLetchie[1] first reported experimental necrosis of the islands of Langerhans. Although most of this report concerns the lesions produced by alloxan, they refer to some experiments in which styryl quinoline No. 90 caused injury of the islands. As the papers cited in this article contained no information concerning the effects on the pancreas, the only report of pancreatic lesions is that observed by them.[1] Since this report, Goldner and Gomori[2] have studied quinoline, cincophen and quinone. These were without effect on the pancreas. Kennedy and Lukens (unpublished) found sublethal doses of quinoline ineffective.

Because of the pancreatic damage ascribed to styryl quinoline, we have examined certain quinoline compounds with respect to their possible pancreatotoxic effect.

Methods. Normal male rabbits were kept in metabolism cages and fed an unmeasured diet of cabbage and oats. Each compound was given (a) intravenously and the response observed for 2-5 days; (b) subcutaneously every day for 2 weeks and (c) in 2 animals one compound was given by stomach tube daily for 2 weeks. The urine was tested daily for sugar, acetone, albumin and specific gravity. Occasional microscopic examinations of the urine sediments were made. The blood glucose was determined once or more on most animals. In taking the blood from the ear, the animals were not trained to this procedure, a fact which presumably accounts for the slightly elevated values obtained. Except for rabbits dying acutely from the drugs or from incidental illnesses, all of them looked well, ate well and maintained their weight. At the end of the experiments they were given nembutal and autopsied. In all cases, the pancreas, liver and kidneys were taken for microscopic examination. In a few animals other organs were sectioned also.

Two compounds were also tested in rats by subcutaneous administration.

The substances tested were as follows:

I 7-chloro-4-(4-diethylamino-1-methylbutylamino)-3-methyl*quinoline,* bis(acid sulfate), hemihydrate.

II 7-chloro-4-(4-diethylamino-1-methylbutylamino)*quinoline,* diphosphate.

III 1-(7-chloro-4-quinolylamino)-3-diethylamino-2-*propanol,* diphosphate.

IV 4-(7-chloro-4-quinolylamino)-α-diethylamino-*o-cresol.*

V 2-[2-(1-amyl-2, 5-dimethyl-3-pyrryl) vinyl]-1,6-dimethyl-quinoliniumchloride.

VI Styryl Quinoline: 2 (p-acetylaminostyryl) 6-dimethylaminoquinoline-methochloride.

VII N-[2-(p-Diethylaminostyryl)-6-methylquinoline] ethochloride.

Results. In the case of compounds I to VI, there was neither physiological nor histological evidence of pancreatic damage. The results have been summarized in Table I. Animals dying in a few minutes have been excluded from this summary.

The kidneys deserve particular mention because of Sheehan's[1] report of nephritis from styryl quinoline. No gross injury was found in any experiments. The slight clouding on the tests for albumin never exceeded that seen in numerous normal rabbit urines. The specific gravity varied widely and included samples with good concentration, all resembling normal rabbits on the same diet.

In addition to the absence of diabetes, there was no evidence at autopsy of any harmful

1 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet,* 1943, **1**, 484.

2 Goldner, M. G., and Gomori, G., *Endocrinology,* 1944, **35**, 24.

TABLE I.
Absence of Diabetogenic Effect of 6 Quinoline Compounds.

Compound (see text)	No. rabbits	Dosage: Amt mg/kg	Dosage: Route*	Blood glucose: No.	Blood glucose: Range mg/100 ml	Days until autopsy (range) days
I	8	10–20	i.v.	10	102-144	3–8
	2	20	oral	6	109-131	14–15
	2	20	s.c.	7	115-153	12–15
II	6	20	i.v.	6	103-121	4–5
	5	20–40	s.c.	12	102-130	15
III	6	20	i.v.	5	105-128	4–5
	4	20	s.c.	8	108-125	14
IV	6	10–20	i.v.	6	107-124	4–6
	6	20	s.c.	14	106-151	11–15
V	2	2–4	i.v.	0		1
	9 rats	4–16	s.c.	5	125-162	4–5
VI	4	10–30	s.c.	0		2

* i.v. = intravenous; s.c. = subcutaneously.

TABLE II.
Effect on Pancreatic Islets of Compound VII: N[2(*p*-Diethylaminostyryl)-6-methyl quinoline] ethochloride.

Animal and No.	No. in group	Doses × days, mg/kg	Time of autopsy, days	Necrosis of islets	Blood sugar at autopsy
		A. Lesions observed.			
Rabbit 70		15 × 1 i.v.*	<1	+++	
" 76		30 × 1 i.v.	<1	+	
Rat 1		150 × 1 s.c.	1	+	
" 3		60 × 1 s.c.	1	+	
" 5		10 × 1 s.c.	1	+	
" 6		10 × 1 s.c.	1	+	
" 10		5 × 1 s.c.	1	+	
" 17		2 × 2 s.c.	1	+	
		B. No lesions present.			
Rabbits	8	10-30 × 1 i.v.	<1 to 5	0	121-178 (5 rabbits)
Rats	12	10 × 1 to 1 × 6 s.c.	1 to 6	0	101-702 (7 rats)

* i.v. = intravenously; s.c. = subcutaneously.

effect on the liver or other organs studied. The lungs, thyroid, thymus, adrenals, spleen were all grossly normal. Microscopic examination showed that the pancreas and islands of Langerhans were entirely normal. The liver, kidney and other organs showed no definite abnormality.

Of 28 animals tested with compound VII, 8 showed evidence of early necrosis of the islands of Langerhans. Table II summarizes these experiments, and Figures 1-4 illustrate the lesions in 2 of these animals. A normal island and the early necrosis from alloxan are presented for comparison. Early necrosis is shown by the shrunken, pyknotic nuclei and by the loss of normal granulation of the cytoplasm. There was no consistent relation of these lesions to dosage; they seemed to be chance occurrences. However, lesions were seen only among the animals which died from the drug. In no instance did an animal survive with diabetes. Because of their early death, blood sugars were not obtained on the animals with lesions. Of those without lesions (series B) the blood glucose values in the rabbits are not regarded as significant. Four

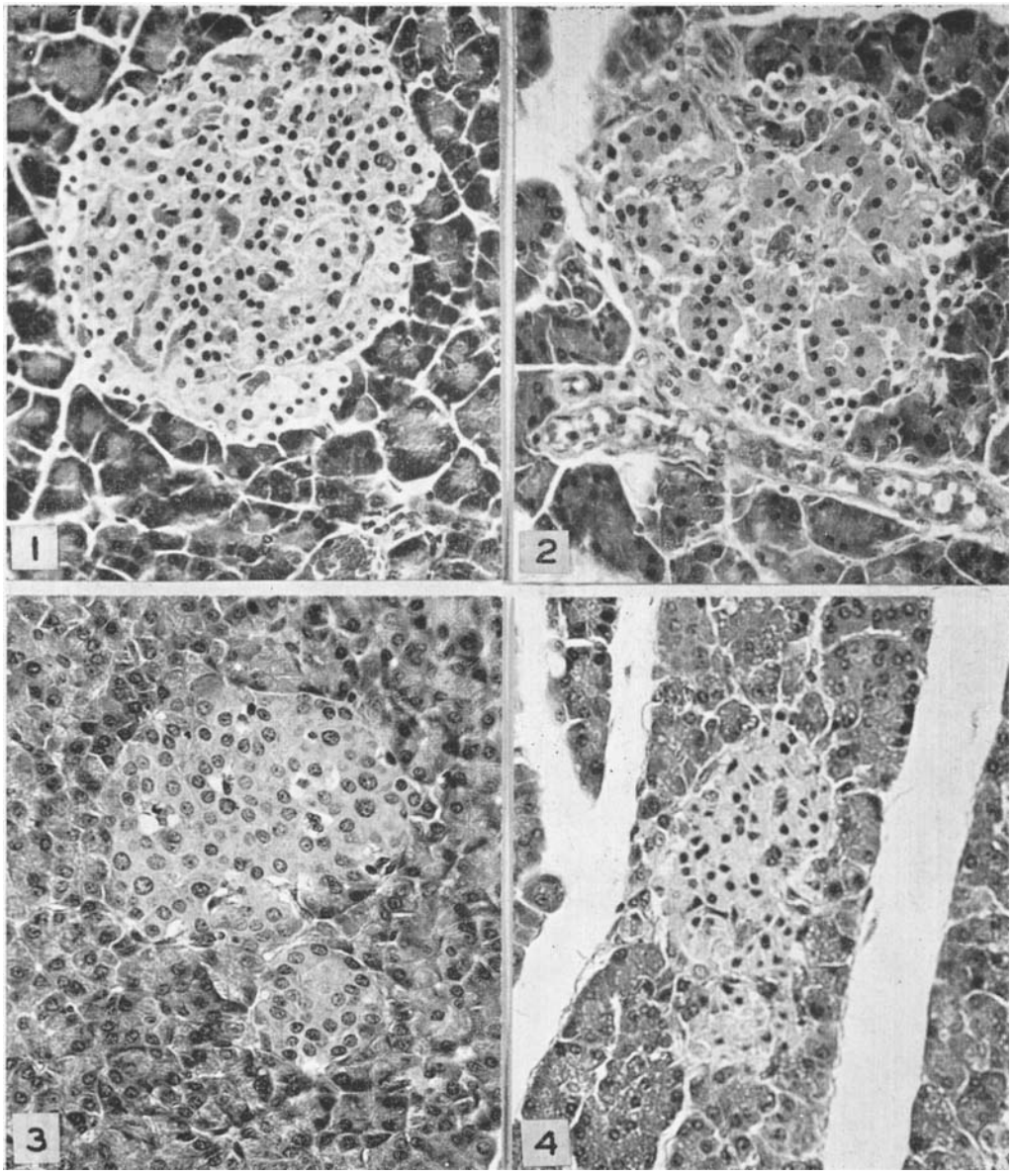

FIG. 1.
Island of Langerhans of Rat 3. Early necrosis is indicated by the pyknotic nuclei and loss of granularity of cytoplasm.

FIG. 2.
Island of Langerhans of Rat 17 showing early necrosis.

FIG. 3.
Normal island (rabbit).

FIG. 4.
Necrosis of island 11 hours after alloxan (rabbit).

of the rats without lesions had very high blood sugars indicative of diabetes but these animals were moribund at the time the blood sugars were determined. In all of 5 rabbits and 7 rats in which the kidney was examined microscopically, there was tubular necrosis of varying severity.

Discussion. The statement that adminis-

tration of compounds I to VI caused no pancreatic damage requires the following comment:

(a) The blood sugars were frequently above the normal level and this has been attributed to the technique used. When normal rabbits were handled in this manner blood sugars have been of this magnitude. There is a clear distinction between these values and those of rabbits with alloxan diabetes in which the blood sugars are characteristically high *i.e.*, 300 to 500 mg per 100 cc. The consistently negative urines provided a mass of evidence, not included in the tables, that these rabbits were not diabetic as a result of the quinoline compounds.

(b) The conclusion that these compounds are not diabetogenic is based on results in a single species. Although the rabbit was used by Sheehan[1] in the study of styryl quinoline, and although the rabbit is the animal most susceptible to alloxan, this limitation must be mentioned.

(c) The tendency to compare other toxic chemicals with alloxan may lead to errors in spite of every effort that has been made to observe diabetogenic activity. Thus, alloxan in similar sublethal doses would have caused diabetes with certainty, within the time limits of these experiments. A more insidious toxin could theoretically escape detection.

As far as we know, the results obtained with compound VII provide the first confirmation of Sheehan's[1] report that styryl quinoline caused injury of the pancreatic islands. As we gave styryl quinoline to only 4 animals our negative results with this compound (VI) might be accidental. This series was small because of Bailey's personal communication that he had not produced lesions of the islands or diabetes with styryl quinoline. The presence of lesions in the larger series of animals given a derivative of styryl quinoline (VII) is in general accord with Sheehan's[1] observations. He described "from memory" the consistent occurrence of islet necrosis after lethal doses of styryl quinoline (VI). In our series lesions occurred in the islets only after lethal doses but their occurrence was quite inconstant. In view of the absence of detailed protocols in the original report[1] further discussion is omitted.

Summary. Six quinoline derivatives have been administered intravenously and subcutaneously to rabbits and rats. There was no functional or anatomical evidence of damage to the islands of Langerhans or to any other organ under the conditions employed.

A seventh compound, N- 2-(p-Diethylaminostyryl)-6-methyl quinoline-ethochloride caused lesions of the islands resembling the early necrosis produced by alloxan. The lesions occurred in 8 of 28 animals tested. Islet necrosis was always associated with lethal dosage but death from the drug was not always accompanied by necrosis. Tubular damage in the kidney was found in all cases examined. Survival with diabetes was not observed.

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Potential Antigenicity of Parenterally Administered Cytochrome C.

L. W. ROTH, R. K. RICHARDS, AND I. M. SHEPPERD.

From the Department of Pharmacology, Abbott Laboratories, North Chicago, Ill.

The separation and purification of cytochrome C by Keilin *et al.*[1] opened the field for its use in animal and human experimentation, in which encouraging results have since been claimed.[2-4] Its production usually involves

[1] Keilin, D., Hartree, E. F., and Hartree, F. R. S., *Proc. Roy. Soc. Med.*, 1937, **122**, 299.

[2] Proger, S., *Bull. New England Med. Center*, 1945, **7**, 1.

[3] Proger, S., and Decaneas, D. J., *Bull. New England Med. Center*, 1945, **7**, 149.

[4] Frank, H. A., Seligman, A. M., and Fine, J., *J. Clin. Invest.*, 1945, **24**, 435.