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THE EFFECT OF *X*-IRRADIATION ON THE RESPONSE OF THE INTESTINE TO ACETYLCHOLINE AND ON ITS CONTENT OF "PSEUDO"-CHOLINESTERASE

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Recently an investigation was published of the effect of *x*-irradiation on the pressor amines in the adrenal medulla (Anderson, Blaschko, Burn, and Mole, 1951). In the course of this work the effect of *x*-irradiation in causing changes in the intestine was noted. The rats suffered from diarrhoea, which indicated increased motility of the intestines, while, on the other hand, when the rats were killed at the end of 72 hr., the lower bowel appeared to be distended and flaccid. We decided to investigate the response of isolated loops of intestine taken from rats after irradiation in order to see if there was any change in their contractile response to acetylcholine and to histamine. We now describe this work in which we found that isolated loops of intestine show changes in response to the stimulant action of acetylcholine after irradiation, but not in response to histamine. Since the effects observed might be due to a change in the amount of cholinesterase, we made determinations of the amounts of this enzyme, and found changes in this also.

METHODS

Irradiation of the rats.—Male albino rats from an inbred laboratory stock were irradiated singly or in pairs in an aluminium box. Treatment factors were 240 kv. 15m. amp. H.V.L. 1.14 mm. Cu ; tube distance 70 cm., dose rate 42 r./min. The field was uniform to $\pm$ 3 per cent. Total dose 1,000 r. The rats weighed from 250 to 350 g., and were usually litter mates.

Reaction of loops of intestine to acetylcholine.—Some of the rats which had been irradiated were killed 24 hr. later, some 48 hr. later, and some 72 hr. later. When an irradiated rat was killed, a control rat was killed at the same time, and the responses of corresponding loops of intestine to acetylcholine and to histamine were compared. From each rat one loop of duodenum was taken, three loops from the jejunum and ileum, and one loop from the colon.

The ileum terminates in the side of the caecum and the distal end of the caecum is continued as a portion marked by oblique striations ; this in turn is continued as a mainly muscular part about 5 cm. long containing the usual faecal pellets which the rat discharges. Under the heading of colon the observations which are recorded were made on the final portion.

Each loop was suspended in an isolated organ bath containing oxygenated Locke's solution at 36° C., and its contractions were recorded. The smallest amount of acetyl-

choline was determined which, when added to the bath, was sufficient to cause a contraction. Similarly, the size of the contraction as recorded on the drum, which was produced by each of three fixed doses of acetylcholine, was determined. In taking loops the length of the unstretched gut was always as near to 4 cm. as possible. The lever was weighted in the same way throughout the experiments, so that the effect of fixed doses of acetylcholine on loops from control and irradiated rats could be compared directly.

As was to be expected, there was variation in the responses of the loops of intestine from different control rats, and it was for this reason difficult to be sure of differences between irradiated and control rats. However, the observations on the jejunum and ileum were made on three loops, so that the mean result given by the three loops of each irradiated rat was compared with that given by the three loops of each control rat. Thus more confidence could be placed in differences observed in the jejunum and ileum than in differences observed either in the duodenum or in the colon. Similar observations to those made with acetylcholine were also made, using histamine.

RESULTS

Change in threshold doses.—The changes in the threshold doses produced by irradiation are shown in Table I, which is a frequency table. Under the heading " duodenum " the threshold doses were between 0.005 and 0.01 μg. in 4 control rats, and between 0.01 and 0.02 μg. in 3 control rats ; finally in 1 control rat it was between 0.08 and 0.16 μg. In rats killed 24 hr after irradiation the threshold dose was between 0.002 and 0.005 μg. in 1 rat, between 0.005 and 0.01 μg. in 1 rat, and between 0.01 and 0.02 μg. in 1 rat.

TABLE I

FREQUENCY TABLE OF THRESHOLD VALUES FOR ACETYLCHOLINE

Each column heading is the dose of acetylcholine (μg.) in a 15-ml. bath. The number under the heading is the number of animals in which the threshold dose was equal to, or less than, the column heading

Irradiation time		0.002	0.005	0.01	0.02	0.04	0.08	0.16
Duodenum	Control	—	—	4	3	—	—	1
	24 hr.	—	1	1	1	—	—	—
	48 hr.	1	1	—	—	—	1	—
	72 hr.	—	1	1	1	1	—	—
		0.002	0.005	0.01	0.02	0.04	0.08	0.16
Jejunum and ileum	Control	—	—	—	—	—	4	4
	24 hr.	—	—	1	—	1	1	—
	48 hr.	—	1	1	1	—	—	—
	72 hr.	—	—	1	1	1	—	1
		0.0013	0.005	0.02	0.08	0.32	More than 0.32	
Colon	Control	—	—	2	3	2	1	
	24 hr.	2	—	—	1	—	—	
	48 hr.	—	1	1	—	1	—	
	72 hr.	—	—	—	1	2	1	

Table I shows quite clearly the lowering of the threshold doses in jejunum and ileum 48 hr. after irradiation. The threshold doses for 8 control rats, from each of which 3 loops were examined, were in the range 0.08–0.16 μg., but those of the irradiated rats were 0.02 μg. or lower. The results in jejunum and ileum at 24 hr. after irradiation already showed some sign of this increased sensitivity, so marked at 48 hr. At 72 hr. there was still increased sensitivity in 3 out of 4 rats.

The differences were less in the duodenum. One out of 3 rats was more sensitive at 24 hr., 2 out of 3 were more sensitive at 48 hr., and 1 out of 4 was more sensitive at 72 hr. In the colon 2 out of 3 were more sensitive at 24 hr., 1 out of 3 at 48 hr., but 0 out of 3 at 72 hr.

Changes in response to fixed doses.—The changes in the response to fixed doses are shown in detail in Tables II and III, in which the results for each irradiated rat are placed beside those of the control rat, which was examined at the same time. Table II shows that at 24 hr. after irradiation there was no increase in the response either in the duodenum or in the jejunum and ileum. There was, however, an increased response in the colon in each of the three experiments. There was considerable variation in the responses of the control rats, the responses being much bigger in Exp. 7 than in Exps. 4 and 8. This variation may have been due to slight differences in the composition of the Ringer's solution or in the temperature or in the degree of oxygenation. Consequently the responses in the irradiated rats should be compared with those of the control rats in the same experiment where such differences did not arise, and not with those of control rats in other experiments.

At 48 hr. after irradiation there was an increased response to all doses of ACh in all three experiments in the duodenum, and in all three experiments in the jejunum and ileum. In the colon there was an increase in two experiments, but a decrease in Exp. 3 (Table II).

At 72 hr. after irradiation the response to fixed doses was less in the jejunum and ileum and colon than in the control rats. The only remaining sign of increased response was in the duodenum, when the lower doses of ACh were used (Table III).

The general picture presented by the results to fixed doses, therefore, was, first, of an increased sensitivity after 24 hr. to be observed in the colon but not elsewhere in the intestine ; second, of an increased sensitivity after 48 hr. to be observed throughout the duodenum, jejunum, and ileum, but not present in all animals in the colon ; third, of a disappearance of this increased sensitivity after 72 hr.

Sensitivity to histamine.—No changes in sensitivity to histamine were observed.

Changes in cholinesterase.—In view of these findings of the change in the response to acetylcholine, we decided to investigate changes in cholinesterase, since the increase in sensitivity to acetylcholine might be due to a diminution in the amount of one of the cholinesterases. Changes in sensitivity to acetylcholine were not always present at 24 hr., and then only in the colon ; we therefore first made examinations at 48 hr. in jejunum and ileum.

Method.—Each rat was killed by a blow on the head, and the carotid arteries were cut. In ileum preparations the upper end of the piece taken was about 7 cm. from the pyloric sphincter ; a piece about 20 cm. long was removed and quickly put into 0.9 per cent saline. This piece was cut into approximately 4 cm. lengths, and these were used alternately for the whole extract and muscle layer. Each piece was slit open and carefully washed with saline ; the surface moisture was removed with filter paper, and

TABLE II

RESPONSES TO FIXED DOSES OF ACETYLCHOLINE 24 HR. AND 48 HR. AFTER IRRADIATION

Part of intestine	Acetylcholine μg.	24 hr. after irradiation			48 hr. after irradiation		
		Exp.	Contraction in mm.		Exp.	Contraction in mm.	
			Control	Irradiated		Control	Irradiated
Duodenum..	0.1	4	10	3	2	6	14
	0.2		15	6		8	25
	1.0		21	11		13	46
	0.1	7	4	6	3	3	19
	0.2		12	11		6	28
	1.0		38	24		10	34
	0.1	8	13	15	5	4	20
	0.2		22	25		8	32
	1.0		48	35		16	47
Jejunum and ileum ..	0.1	4	2	2	2	2	7
	0.2		3	3		3	8
	1.0		5	5		4	12
	0.1	7	3	6	3	1	9
	0.2		6	12		3	11
	1.0		11	22		6	17
	0.1	8	2	3	5	2	7
	0.2		3	4		5	11
	1.0		4	7		10	16
Colon ..	0.1	4	0	5	2	0	13
	0.2		0	10		0	23
	1.0		2	38		4	35
	0.1	7	18	53	3	2	0
	0.2		36	61		3	0
	1.0		52	100		15	2
	0.1	8	7	26	5	2	28
	0.2		9	41		17	45
	1.0		24	60		34	76

the tissue weighed. In the extracts of muscle coat alone the mucous membrane was removed with a scalpel until a more transparent layer was left. The tissue was put into a previously cooled mortar, cut up with scissors, and quickly frozen to $-15°$ C. When it was completely frozen it was ground up until it thawed again. A small amount of silica was added to complete the grinding, and 10 ml. of Krebs's bicarbonate Ringer was added to each gramme of tissue. The extracts were stored at $-15°$ C. until used. In the later experiments the extracts, prepared as above, were finally placed in a small glass homogenizer to obtain a more uniform extract ; the results obtained with these extracts were more consistent than the earlier ones.

The extracts of colon were made in the same way ; a strip from below the obliquely striated part was taken ; observations were made from whole extracts only in this region, owing to lack of sufficient material to study muscle separately.

TABLE III

RESPONSES TO FIXED DOSES OF ACETYLCHOLINE 72 HR. AFTER IRRADIATION

In Exp. 1 the letters (a), (b), and (c) indicate strips from different rats

Part of intestine	Exp.	Acetylcholine μg.	Contraction in mm.	
			Control	Irradiated
Duodenum	1	0.1	3	(a) 9, (b) 9 } 9
	6		4	6
	1	0.2	5	(a) 18, (b) 14, (c) 30 } 21
	6		7	8
	6	1.0	15	11
Jejunum and ileum ..	1	0.1	4	(a) 7, (b) 8, (c) 3 } 6
	6		3	2
	1	0.2	7	(a) 9, (b) 10, (c) 4 } 8
	6		7	4
	1	1.0	15	(a) 10, (b) 14, (c) 5 } 12
	6		30	21
Colon	1	0.1	10	(a) 2, (b) 1, (c) 11 } 5
	6		7	0
	6	0.2	11	0
	1	1.0	30	(a) 13, (b) 10, (c) 41 } 21
	6		17	3

The activity of the preparation was measured by the Warburg technique ; all experiments were repeated, either at the same time (if the flasks were being duplicated) or in a separate experiment. The activity was expressed as millilitres of CO_2 evolved per hour per gramme of wet tissue. The substrates used were acetylcholine bromide, benzoyl choline chloride, and acetyl-β-methylcholine chloride (mecholyl).

Both substrates and enzyme extracts were made up in Krebs's bicarbonate Ringer, and the gas mixture used was 95 per cent nitrogen, 5 per cent CO_2.

The flasks were set up as shown in Table IV ; the spontaneous hydrolysis of the substrate was not determined in the routine experiments, as it would not affect direct comparisons between control and irradiated rats. Acetylcholine and benzoylcholine were used in a final concentration of 0.015 molar, and acetyl-β-methylcholine in a concentration of 0.03 molar.

TABLE IV

ARRANGEMENT OF MANOMETER EXPERIMENTS

Thermo-barometer	Enzyme blank I	Reaction I	Enzyme blank II	Reaction II	Enzyme blank III	Reaction III	
3.0 ml. water	0.2 ml. extract (=20 mg.) +2.5 ml. bicarbonate Ringer		0.4 ml. extract (=40 mg.) +2.3 ml. bicarbonate Ringer		1.0 ml. extract (=100 mg.) +1.7 ml. bicarbonate Ringer		Main flask
—	0.3 ml. bicarbonate Ringer	0.3 ml. 0.15M ACh bromide	0.3 ml. bicarbonate Ringer	0.3 ml. 0.15M benzoyl-choline chloride	0.3 ml. bicarbonate Ringer	0.3 ml. 0.3M acetyl-β-methyl-choline	Side bulb

Results at 48 hr.—When the substrate used was either acetylcholine or benzoylcholine, the activity of the irradiated tissue at 48 hr. was in every experiment appreciably less than that of the control tissue (see Table V). The mean results for the irradiated tissue were all between 40 and 65 per cent of the control results.

There was no similar change in the irradiated tissue when acetyl-β-methylcholine was used as a substrate ; the mean result was 103 per cent for the whole intestine and 124 per cent for the muscle coats only. The conclusion can therefore be drawn that, both in the irradiated intestine taken as a whole and the muscle coats alone, there

TABLE V

CHOLINESTERASE ACTIVITY AT 48 HR. IN JEJUNUM AND ILEUM

Substrate	ml. CO_2 per g. wet tissue per hr.			
	Whole gut		Muscle only	
	Control	Irradiated	Control	Irradiated
Acetylcholine	14.4	9.2	—	—
	20.3	8.6	13.6	8.4
	18 8	5.7	13.1	9.1
	16.1	8.0	10.2	8.7
	12.8	4.7	14.5	6.7
Mean ..	16.5	7.2	12.8	8.2
Benzoylcholine ..	5.3	2.2	—	—
	5.4	2.1	3.2	1.6
	4.1	1.4	2.8	1.5
	4.8	2.4	3.2	1.4
	3.6	1.3	4.3	2.2
Mean ..	4.6	1.9	3.4	1.7
Acetyl-β-methylcholine	0.9	1.3	—	—
	1.6	1.5	1.0	1.7
	2.1	1.1	1.4	1.7
	2.1	1.4	1.4	1.6
	0.5	0.9	1.1	1.1
Mean ..	1.4	1.2	1.2	1.5

was a consistent fall in the "pseudo" or "non-specific" cholinesterase, but no similar change in the "true" or "specific" cholinesterase.

Table V shows considerable variation in the figures for whole gut when acetyl-β-methylcholine was used. This variation may have been due to the variable destruction of "true" cholinesterase by the proteolytic enzymes present in the mucosa.

Changes at 24 hr.—A similar number of experiments were carried out 24 hr. after irradiation, the jejunum and ileum being examined in suspensions of the whole intestine and also of the muscle coats only; a suspension of the whole colon was also examined. We expected to find no change in the cholinesterase present in the jejunum and ileum, but we expected a fall in the cholinesterase present in the colon. In fact there appeared to be a slight change in both parts of the intestine. In Table VI the mean results of seven experiments are given.

TABLE VI

CHOLINESTERASE ACTIVITY OF IRRADIATED TISSUES

Mean results of seven experiments after 24 hours' irradiation

Part of intestine	Cholinesterase activity as percentage of control for different substrates					
	Acetylcholine		Benzoylcholine		Acetyl-β-methylcholine	
	Whole	Muscle	Whole	Muscle	Whole	Muscle
Jejunum and ileum ..	85	102	87	101	143	128
Colon	94	—	85	—	115	—

The figures in Table VI suggest some fall in the "pseudo" or "non-specific" cholinesterase when a suspension of the whole intestine was used, but not when a suspension of muscle coats only was used. The fall is, however, slight, and little importance would be attached to it but for the evidence of the fall at 48 hr., which adds probability to the existence of a smaller fall at 24 hr.

Discussion

The experiments described in this paper were undertaken because observation of the alimentary tract of irradiated rats showed that it was affected by the irradiation. By examining strips of intestine in an isolated organ bath we found that at the end of 24 hr. the colon gave a supernormal response to acetylcholine, and that at the end of 48 hr. the duodenum, the jejunum, and the ileum showed a similar change. At 48 hr. the increased responsiveness of the colon was no longer seen in some of the rats.

Since we observed no corresponding changes in the reaction of the gut to histamine, which might have indicated an increased excitability to all stimuli, we examined intestinal loops of irradiated rats for the two forms of cholinesterase, because it was clear that an increased responsiveness to acetylcholine alone might be due to a diminution in cholinesterase. We found a diminution in the amount of "pseudo" or "non-specific" cholinesterase in all pieces of jejunum or ileum examined 48 hr. after irradiation, the drop being to about half the value in the controls. The fall in cholinesterase was evident not only in suspensions prepared

from the whole intestine, but also in those prepared from the muscle coat only. There was no change in the amount of " true " or " specific " cholinesterase. When strips of colon or of ileum were examined 24 hr. after irradiation, the mean results indicated a slight fall in the " pseudo " cholinesterase present in suspensions of both colon and ileum, but not in suspensions of muscle coat only.

Acetylcholine is continuously produced in the wall of the intestine by choline-acetylase of non-nervous origin, as Feldberg and Lin (1950) have shown. The function of this acetylcholine is, they say, to provide a background of tone and rhythmic movements necessary for initiation of the peristaltic reflex, and also to act as a physiological stimulus for the continuous secretion of succus entericus. The correlation between the increased responsiveness of strips of intestine to acetylcholine and the fall in " pseudo "-cholinesterase which we have found is strong evidence that in the intestine it is " pseudo " or " non-specific " cholinesterase which destroys the acetylcholine which is formed. Recently Koelle, Koelle, and Friedenwald (1950) have come to the same conclusion as a result of work on the isolated cat's ileum, in which they used di*iso*propylfluorophosphonate (DFP) to inhibit cholinesterase. They determined the degree of inhibition by histochemical methods, and they found that concentrations of DFP having little or no " effect on specific ChE (cholinesterase) but markedly inhibiting non-specific ChE (cholinesterase) produced increases in tonus and occasionally in the amplitude of contractions."

How irradiation reduces " pseudo "-cholinesterase in the intestine we cannot say. Changes in the amount of " pseudo " or " non-specific " cholinesterase in various organs, particularly the liver, and the plasma of rats, have been described by various workers. Everett and Sawyer (1946a) demonstrated that ovariectomy caused a rapid decline in the content of this enzyme in the serum to a level a little above that found in males, whereas castration in the male caused a rise up to this level. When an oestrogen was given there was a rise in the serum cholinesterase activity, whereas testosterone caused a fall. Sawyer and Everett (1947) concluded that the liver was the main site of synthesis of " non-specific " cholinesterase, and they discussed its distribution in different organs. The importance of the thyroid hormone was shown by Hawkins, Nishikawara, and Mendel (1948). They found that thyroidectomy doubled the amount of pseudocholinesterase in male rat plasma, and that the administration of thiouracil almost trebled it. Thyroxine administration, on the other hand, reduced it to about 70 per cent of the normal amount. These changes occurred without any corresponding change in " true " cholinesterase. Everett and Sawyer (1946b) showed that the effect of oestrogens was exerted by way of the anterior lobe of the pituitary, and it is possible that the changes in " pseudo " or " non-specific " cholinesterase which follow changes in the amount of thyroid hormone are also secondary to an effect on the anterior lobe.

If " pseudo " or " non-specific " cholinesterase is synthesized in the liver, it seems likely that the effect of irradiation may be primarily on the liver. If the synthesis in the liver is controlled by the anterior pituitary, the effect of irradiation may be part of the reaction of the anterior pituitary to the " stress " produced by the irradiation.

In conclusion it may be noted that Harrison and Brown (1951) have studied the effect of starvation on the pseudocholinesterase activity of the liver and serum of rats ; they found that in female rats there was a rapid fall in the liver even in so short a

period as 24 hr., and a further fall at the end of 48 hr. In male rats, however, there was very little change, and in the present investigation only male rats were used.

SUMMARY

1. Since *x*-irradiation causes obvious changes (e.g., diarrhoea) in the intestinal tracts of rats, an investigation was made of the responses of isolated loops of intestine taken from irradiated rats to acetylcholine and histamine.

2. Increased responses were observed to acetylcholine in the colon 24 hr. after irradiation. Increased responses were likewise observed in the duodenum, jejunum, and ileum after 48 hr. No similar changes in response to histamine were observed.

3. The cholinesterase activity was determined in loops of intestine from irradiated rats. After 48 hr. the amount of " pseudo " or " non-specific " cholinesterase was found to have fallen to about half in the jejunum and ileum, this being true of suspensions of the whole gut and of muscle coats only. There was no fall in " true " or " specific " cholinesterase.

4. Some fall in ".pseudo "-cholinesterase was already evident in both colon and ileum 24 hr. after irradiation.

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