Effects of 5-Fluorouracil on Exocrine Glands
I. Gland Weights in Mice Receiving Synthetic Polynucleotides (36340)

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Since the early advocacy by Heidelberger *et al.* (1) of using fluorinated pyrimidines as tumor-inhibiting compounds, the nucleotide analogue, 5-fluorouracil (5-FUR), has been used in a number of experiments designed to study its effect on DNA synthesis via blockade of uracil methylation (2), or RNA synthesis through competition with the replacement of the natural nucleotide (3), and on protein synthesis via the blockade of more complex cellular processes (4).

With respect to digestive glands, Martin and Levin (5) studied the structural effects of 5-FUR administration on pancreatic acinar cells, while Stenram (6) studied the fine structure, together with RNA and protein synthesis in liver cells of rats injected with 5-FUR. Earlier, Kuglar *et al.* (7) observed an enlargement of the pancreas in 5-FUR-treated animals, which they considered to be due to a blocking action upon the discharge mechanisms of acinar cells. Similar observations have been made in rat pancreas following administration of actinomycin and other antimetabolites (8).

Although the precise mode of action remains to be demonstrated, synthetic polynucleotides have recently been used to enhance antibody protein synthesis and interferon production (9).

In order to determine if the adjuvant action of the synthetic polynucleotides on antibody production might be due to a general effect upon protein biosynthesis, we decided to study the effects of the synthetic polynucleotide, a homopolymer of polyadenylic and polyuridylic acids (Poly A:U), on RNA and protein synthesis by digestive glands in normal, as well as 5-FUR-treated animals.

This report represents the first in a series of articles on the actions of 5-FUR and poly A:U on digestive glands, and communicates the results from an extensive gland weight study which indicates that an appropriate dose of poly A:U, given to mice treated with sublethal doses of 5-FUR, stimulates the release of secretory product during the recovery period of the glands. This effect is contrasted with results obtained after pilocarpine injection which, in the pancreas, failed to stimulate the 5-FUR blocked release.

Materials and Methods. A total of 128 adult male mice of Balb C/strain were used. Of these, 20 mice were used in a dose–response study in which 5 mice each were given three daily intramuscular injections of 10, 25, 50, or 100 mg/kg of 5-FUR in 0.1 ml of physiologic saline. Following the last injection, the animals were weighed daily for 10 days. The results from this study showed that moderate, but significant, changes in weight were observed with 3 injections of the 25 mg/kg dose (Table I).

In the next experimental series, 108 mice were paired according to weight, and were divided into three groups of 36 animals each. In each group one of the pairs was given 3 daily injections of 25 mg/kg of 5-FUR; whereas the other mouse was given a comparable amount of the vehicle alone. All animals were pair-fed throughout the study, and were fasted for 24 hr before sacrifice.

The groups were: (I) 5-FUR alone; (II) 5-FUR plus pilocarpine; and (III) 5-FUR plus poly A:U. Three pairs of animals of group I were sacrificed on days 1, 3, 5, 7, 10, and 14 following the last injection. Animals were sacrificed by cervical dislocation following which body and organ weights of the major salivary glands and pancreas were

TABLE I. Changes in Body Weights (g) of Mice After Different Doses of 5-FUR Injections.[a]

Day[b]	3 × 10 mg/kg			3 × 25 mg/kg			3 × 50 mg/kg			3 × 100 mg/kg		
	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control
1	24.2 (1.6)	0.09	93.0	23.7 (4.4)	0.06	91.1	24.0 (2.5)	0.05	92.3	22.2 (1.0)	0.001	85.3
2	23.8 (2.1)	0.04	89.8	22.2 (1.7)	0.01	83.7	21.5 (1.6)	0.001	81.1	20.3 (1.1)	0.001	76.5
3	24.7 (2.2)	0.05	92.1	21.0 (1.6)	0.001	78.6	20.5 (2.4)	0.001	76.7	19.1 (1.1)	0.001	71.5
4	25.6 (1.5)	0.09	95.1	20.8 (2.6)	0.001	77.6	19.8 (1.8)	0.001	73.6	18.1 (1.4)	0.001	67.2
5	26.0 (2.1)	0.3	96.6	20.5 (1.8)	0.001	76.2	19.3 (4.5)	0.001	71.7	17.9 (2.8)	0.001	66.7
6	26.5 (2.2)	0.5	97.7	21.6 (2.1)	0.001	79.7	20.7 (2.1)	0.001	77.1	17.4 (1.3)	0.001	64.2
7	26.8 (2.4)	0.5	98.8	22.5 (2.0)	0.008	83.0	21.4 (2.0)	0.004	78.9	17.1 (1.6)	0.001	63.1
8	26.9 (1.8)	0.4	100.3	23.4 (1.9)	0.01	87.3	22.2 (2.5)	0.009	82.8	17.0[c]		63.4
9	26.8 (1.9)	0.5	99.6	24.5 (1.9)	0.04	91.0	22.7 (3.2)	0.01	84.3			
10	26.7 (2.2)	0.5	99.6	25.3 (1.2)	0.08	94.4	23.9 (3.1)	0.02	89.5			

[a] Three daily injections were given.
[b] Day after the last injection.
[c] Only 1 out of 5 mice survived.

immediately determined. Animals in group II received two injections of 0.5 mg/kg of pilocarpine, 2 and 4 hr prior to sacrifice, and 3 pairs were sacrificed at each of the same intervals as in group I. The animals of group III received a single injection of 100 μg of poly A:U 24 hr prior to sacrifice, and were sacrificed as in groups I and II. The amount of poly A:U used in this experiment is that amount which, given in immunologically challenged mice of the same strain, would produce a significant enhancement of circulating antibody titer. The results were evaluated statistically by calculating the standard deviation of arithmetic means and comparing the control and experimental values within an interval by the Student's t test.

Results. Table I records the body weight changes observed in the preliminary dose study. It indicates that, with 3 daily injections of 100 mg/kg dose, few animals survived after the first week; whereas only a minor and transient reduction of body weight occurred after 3 similar injections of 10 mg/kg of 5-FUR. On the other hand, a dose of 3 × 25 mg/kg of 5-FUR produced nearly 25% difference in body weights on day 5 with definite signs of recovery on day 6 or 7. A similar, but slightly more severe, response was observed with the 3 × 50 mg/kg dose. The p values indicated reflect the differences between the experimental and paired controls within each group at given time periods.

Table II presents the changes of body weights from the second experiment using 3 × 25 mg/kg dose with or without pilocarpine on poly A:U. As shown, the general pattern of body weight change is similar among the 3 groups, showing a low on day 5 and a recovery of 86% or more of the control by day 14.

Tables III through V show gland weights of the 3 groups which, unlike body weights, were significantly different from one another. Changes in gland weights of mice injected with 5-FUR alone (group I) appear in Table III. There was a steady increase of digestive gland weights during the first 5 days; *e.g.*, during the time when the body weights decreased continuously. From day 5 and on there was a clear tendency towards reversal of gland weight changes, which returned to

TABLE II. Body Weights (g) of Mice Injected with 5-FUR or 5-FUR Plus Poly A:U or Pilocarpine.[a]

	5-FUR				5-FUR + Pilocarpine				5-FUR + Poly A:U			
Day[b]	Mean ± (SD)		p	% of control	Mean ± (SD)		p	% of control	Mean ± (SD)		p	% of control
1	18.3	(0.6)	0.03	84.7	22.6	(1.4)	0.06	88.3	17.2	(1.1)	0.07	83.1
3	16.1	(0.7)	0.001	74.5	20.1	(1.1)	0.001	78.5	15.7	(1.4)	0.01	75.7
5	14.1	(0.4)	0.001	65.3	18.4	(1.3)	0.001	71.9	13.8	(1.7)	0.001	66.7
7	15.6	(1.1)	0.001	72.2	20.9	(1.6)	0.001	81.6	14.4	(1.6)	0.001	69.6
10	17.1	(0.8)	0.01	79.2	22.2	(1.2)	0.005	86.7	16.8	(0.8)	0.001	81.2
14	18.7	(1.7)	0.01	86.6	24.7	(1.6)	0.09	96.5	18.2	(1.8)	0.01	87.9

[a] Agents were used in the following doses: 5-FUR, 3 daily injections of 25 mg/kg; poly A:U, 30 mg/kg given 24 hr prior to sacrifice; and pilocarpine, 2 injections of 0.5 mg/kg each given at 2 and 4 hr prior to sacrifice.

[b] Day after the last injection of 5-FUR.

values that were insignificant from those of the respective control animals by day 14. However, the gland weights of experimental animals were consistently and significantly higher than those of appropriate controls through day 10. Moreover, the parotid and sublingual glands showed a greater increase of weight, reaching over 190% of the control values on day 5. On the other hand, the submandibular gland and pancreas increased to about 130% of the control value on the same day. Thus the changes in body and organ weights take an inversely related course. Considered together, they suggest the possibility of either a nonspecific hydration of the experimental glands or their failure to release secretory products.

The organ weights of mice in group II, *i.e.*, those injected with pilocarpine prior to sacrifice, are shown in Table IV. Weights of the major salivary glands in this group showed only limited differences between experimental and control mice. In most cases, the differences between them were less than 10% of the control and p values between the experimental and appropriate control animal were generally insignificant. In contrast to the salivary glands, the weight of pancreas from the experimental animals was 10 to 21% heavier than that of controls from days 3 through 10. Furthermore, the difference was of uniform significance at the level of .001.

Effects of poly A:U on gland weights observed in mice of group III are shown in Table V. The parotid and sublingual glands of the experimental mice, which in group I showed a maximum of over 190% of the control values, weighted only 120% or so of the control glands, the difference between the experimental and control mice was consistently significant only on day 5. The submandibular gland and pancreas, which in group II were less enlarged, showed also a reduced difference between the experimental and control animals, again being consistantly significant only on day 5.

Discussion. The results of this study, together with histologic evidence from earlier studies (7, 8), indicate that digestive glands may fail to discharge significant amounts of secretory materials in mice that have been injected with 5-FUR. It is evident further that such a retention of products may vary according to the gland. This may not necessarily be related to the type of glandular products, since the mucous sublingual and serous parotid glands showed an equally high level of retention after 5-FUR. That the increase in gland weights is probably due to the blockade of release of secretory products is reinforced by results from the pilocarpine-treated animals. The failure of the pancreas to respond similarly supports earlier observations (5, 7) with respect to the difficulty encountered in zymogen granule and pancreatic lipase release among 5-FUR-treated rats.

In contrast to the complete reversal of salivary gland weights produced by pilocar-

TABLE III. Changes in Glandular Weights (mg) of Mice Injected with 5-FUR Alone (group I).[a]

	Parotid			Submandibular			Sublingual			Pancreas		
Day[b]	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control
1	38.6 (2.5)	0.04	121.0	81.6 (3.1)	0.02	115.6	29.0 (2.0)	0.01	134.2	199.3 (5.0)	0.09	102.1
3	56.7 (3.4)	0.001	177.7	90.6 (5.1)	0.003	128.5	37.6 (1.5)	0.002	174.0	238.6 (6.0)	0.02	122.2
5	61.3 (2.4)	0.001	192.1	97.0 (2.6)	0.001	137.5	42.0 (6.1)	0.001	194.4	254.1 (10.4)	0.001	130.2
7	50.3 (2.4)	0.001	157.6	89.3 (1.5)	0.001	126.6	36.0 (7.3)	0.008	166.6	232.6 (17.2)	0.03	124.3
10	43.3 (4.4)	0.003	135.7	83.3 (7.6)	0.01	118.1	30.0 (4.0)	0.001	138.8	220.4 (16.2)	0.05	112.9
14	33.6 (3.3)	0.1	105.3	70.3 (3.1)	0.3	99.7	24.3 (3.8)	0.1	112.5	202.8 (7.4)	0.2	103.8

[a] The same dose as indicated in Table II.
[b] Day after the last injection.

pine injection, the results from group III, given poly A:U in amounts appropriate for enhancement of antibody synthesis, are of interest in that all of the glands from experimental mice in group III are significantly heavier than controls on day 5.

The discrepancy between groups II and III clearly indicate that poly A:U is not capable of accomplishing an effect analogous to that produced by pilocarpine at times when the destructive effect of 5-FUR is at the maximum. However, the lack of a statistically meaningful difference on days 10 and 14 in all organs suggests that, during the recovery phase, poly A:U is effective in facilitating discharge at the physiologic level. It further suggests that regenerating cells might respond better to poly A:U stimulation than more seriously damaged ones. Whether the poly A:U effect observed herein represents a strictly pharmacologic effect through membrane-mediated stimulation of secretory process, or whether it involves more complicated cellular processes cannot be concluded from this study. Our results indicate, however, that an injection of poly A:U (24 hr prior to sacrifice) produces a discharge effect similar to that of pilocarpine. Recent unpublished results from our laboratory have also demonstrated that poly A:U can directly induce a release of secretory granules, as well as stimulating the biosynthesis of secretory enzymes.

The discrepancies in organ weights between pilocarpine and poly A:U-treated groups provide another line of evidence for the possibility that the release processes may be considerably different between the pancreas and the salivary glands. Within the context of the present study, such differences could be accounted for by any one or more of the following possibilities: (i) the cellular mechanism of release by the exocrine pancreas might be totally different from that of salivary glands; (ii) poly A:U might have different affinities for the glands studied; (iii) poly A:U effects on the pancreatic release mechanisms(s) may be greater than to those of salivary glands; (iv) neural mechanisms regulating the release of secretion products might be different among the glands.

TABLE IV. Changes in Glandular Weights (mg) of Mice Injected with 5-FUR and Pilocarpine (group II).[a]

Day[b]	Parotid			Submandibular			Sublingual			Pancreas		
	Mean ± (SD)		% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control
1	27.2	(1.3)	98.9	49.3	(3.7)	90.2	11.1	(0.7)	79.7	203.7	(18.2)	106.8
3	25.6	(1.8)	91.6	50.4	(4.8)	92.0	10.3	(0.8)	72.4	221.4	(19.4)	116.3
5	26.4	(1.6)	95.2	49.1	(4.3)	92.0	12.5	(1.0)	86.9	230.1	(18.3)	121.0
7	26.1	(2.1)	95.2	48.7	(4.2)	88.4	15.4	(1.2)	97.1	212.8	(18.5)	111.5
10	25.3	(1.9)	91.6	50.5	(4.1)	92.0	13.3	(1.1)	94.2	208.6	(17.1)	109.5
14	27.8	(1.9)	98.9	54.3	(3.8)	99.4	13.6	(1.0)	94.2	191.3	(16.2)	100.5
	0.3			0.2			0.1			0.1		
	0.1			0.2			0.1			0.001		
	0.2			0.1			0.3			0.001		
	0.2			0.1			0.3			0.001		
	0.1			0.1			0.2			0.001		
	0.4			0.3			0.5			0.05		

[a] The same regimen as indicated in Table II.
[b] Day after the last injection of 5-FUR.

Further comparative studies of the fine structure of glands, as well as the fate of labeled poly A:U in these organs, would be of additional value in assessing the nature of our observation.

The lack of differences in gland weights between the experimental and control on day 1 might be due to an entirely different reason. Poly A:U, introduced during this early destructive phase by 5-FUR, may indeed compete with the latter and therefore produce a reduced 5-FUR effect. This is in contrast to earlier findings by Wilson *et al.* (10) who reported an enhancement of teratogenic effect of 5-FUR by addition of natural pyrimidines in pregnant rats. This was thought to be due to slowdown of 5-FUR degradation in the liver resulting from competition between the analogue and natural pyrimidines introduced into the system. Further biochemical and ultrastructural studies in progress will clarify the nature of poly A:U effects on digestive gland functions of animals in which experimental tumor growth is arrested by 5-FUR.

Summary. Body weights and weights of major salivary glands and pancreas of mice given sublethal doses of 5-FUR were studied after pilocarpine or poly A:U injection. Body weights showed a significant decrease during the first 5 days after 5-FUR alone, while in the same period the glandular weights increased in all 4 organs. Injections of pilocarpine prior to sacrifice abolished the increase in glandular weights in salivary glands, but the pancreas failed to respond. This increase in organ weight was thought to be due to the inability of the organs to release synthesized secretory products. An injection of poly A:U 24 hr prior to sacrifice produced a uniform release of secretory products during the recovery phase in all of the organs studied. Such effect was notably absent on day 5 when the 5-FUR effect was at a maxium. It is concluded that the introduction of synthetic poly A:U might improve the recovery processes following nucleotide insult, as well as trigger release mechanisms in a manner which is different from that of pilocarpine.

1. Heidelberger, C., Chaudhuri, N. K., Danneberg,

TABLE V. Changes in Glandular Weights (mg) of Mice Injected with 5-FUR and Poly A:U (group III).[a]

	Parotid			Submandibular			Sublingual			Pancreas		
Day[b]	Mean ± (SD)		p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control	Mean ± (SD)	p	% of control
1	35.0	(6.0)	0.3	111.4	74.3	(9.0)	0.05	107.9	20.2	(2.5)	0.4	97.5	193.0	(7.5)	0.5	102.2
3	37.3	(4.0)	0.3	118.7	76.0	(7.6)	0.001	110.4	23.0	(3.6)	0.4	111.1	196.3	(7.5)	0.02	103.4
5	39.1	(3.8)	0.01	124.5	77.6	(6.7)	0.001	112.7	25.3	(4.6)	0.03	122.2	198.4	(11.5)	0.001	105.0
7	35.0	(4.0)	0.1	111.4	74.5	(8.7)	0.002	108.2	23.0	(3.6)	0.2	111.1	193.0	(8.3)	0.1	103.4
10	33.6	(4.4)	0.1	107.0	72.4	(5.0)	0.1	105.2	21.6	(3.1)	0.3	104.3	191.6	(9.0)	0.2	101.4
14	31.2	(4.5)	0.3	99.3	70.1	(5.1)	0.2	101.8	18.6	(2.5)	0.2	89.8	189.3	(12.2)	0.1	100.2

[a] The same regimen as indicated in Table II.
[b] After the last injection of 5-FUR.

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