TABLE II.
Comparison of Hyoscine-Benadryl Mixture with Hyoscine Alone.

Preflight medication	Subjects	Airsick (vomited) No.	%
Hyoscine	135	26	19.3
Hyoscine-Benadryl	135	14	10.3

that with Dramamine. Slight protection was given by 8-chlorotheophylline. It is interesting to note that in those instances where Benadryl was unable to prevent vomiting the time of onset was nevertheless delayed over that of the placebo group (34.2 to 29.0 minutes).

Hyoscine hydrobromide has long been the drug of choice in airsickness(4). Because of the difference in pharmacological action of hyoscine and Benadryl, it was thought that a combination of these compounds might be more effective than either alone. Therefore, one hour before flight hyoscine hydrobromide alone was given to one group of subjects in 0.65 mg doses, while a mixture of 0.65 mg hyoscine hydrobromide and 50 mg Benadryl was given to a second group.

Table II indicates the superiority of the mixture. Only 10.3% of the subjects in this group became sick, compared with 19.3% with hyoscine alone, approximately 30% with Benadryl or Dramamine and over 50% with placebos. In every instance significance has been determined between the control and experimental groups by means of a sequential test of hypothesis. The 8-chlorotheophylline series was truncated after 13 flights so that the level of significance is probably between 7 and 10%. In all other cases truncation was not necessary and the difference between the groups was significant at the 5% level. The incidence of vasomotor disturbances and nausea not leading to vomiting was similarly decreased among those subjects receiving hyoscine-Benadryl. The average time of onset of vomiting was delayed from 29.0 minutes with hyoscine alone to 42.0 minutes with the hyoscine-Benadryl combination. Drowsiness and dryness of the mouth were the chief complaints of those receiving the mixture.

Experiments are under way to test smaller doses and other antihistaminics purported to have fewer side effects.

Summary. No significant difference was found between Benadryl (50 mg) and Dramamine (100 mg) in protecting subjects from airsickness during a turbulent flight in an airplane. 8-Chlorotheophylline (50 mg) gave only slight protection as compared with placebos. A combination of hyoscine hydrobromide (0.65 mg) and Benadryl (50 mg) was found to be more effective than either of these drugs alone.

4. Smith, P. K., USAF Sch. Aviat. Med. Res. Proj. 468, Rep. No. 1, June 14, 1946.

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Occurrence and Distribution of an Inhibitor for Desoxyribonuclease in Animal Tissues.* (17634)

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The presence of a specific inhibitor for desoxyribonuclease in the crop gland of brooding pigeons has recently been described(1). The purpose of the present work was to investigate the distribution of the inhibitor in

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several animal tissues, bearing in mind that it was first found in rapidly growing epithelium.

Experimental. Desoxyribonucleic acid was prepared from calf thymus according to Hammersten(2). Once recrystallized desoxyribonuclease (DRNase) was prepared according to Kunitz(3). All experiments were made on the same batch of DRNase. The viscosimetric determination of activity was performed according to Laskowski and Saidel(4), with the exception that variable amounts of inhibitor were added to the reaction system. Unless otherwise specified, the fresh tissue to be examined was ground in the Waring blendor with 10 volumes of water and centrifuged in the cold at 4,000 r.p.m. for 20 minutes.

Early in the course of this work it became apparent that the relationship between the amount of inhibitor and the observed inhibition was not linear. In order to secure even a roughly quantitative method for the determination of the inhibitor, it was necessary to construct an empirical curve. Several attempts in this direction were made, first using the crude extract of veal liver, later a somewhat purified inhibitor from bull testes. Since the same type of phenomenon was observed in both cases, only the results of the latter experiment are presented in Fig. 1. It was arbitrarily decided to call one unit of the inhibitor the amount present in 1 ml of the solution which was used in the experiment shown in Fig. 1. The amount of the inhibitor in an unknown solution was read from the curve, using only its initial part (between 0 and 0.5 ml). The shape of the approximated curve resembled the one found by Kunitz(5) for the inhibition of chymotrypsin α by soybean trypsin inhibitor. It was strikingly different from the direct proportionality found for the system: trypsin, soybean trypsin inhibitor(5). This suggests that the re-

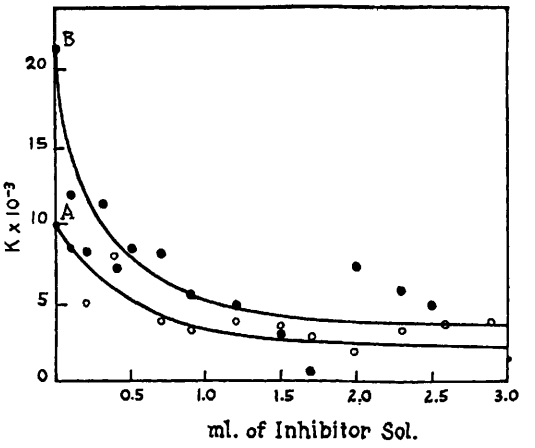

FIG. 1.

The effect of increasing amounts of inhibitor on the activity of desoxyribonuclease. Ordinate: activity of desoxyribonuclease expressed as K (from $K = 1/t \log \frac{\eta_0}{\eta_t}$), abscissa: ml of inhibitor solution added to the viscosimeter. The solution of inhibitor contained 5.0 mg protein per ml. It was obtained by extracting bull testes with 3 volumes of water, fractionating between 30-60% saturation of ammonium sulfate, and finally, dialyzing. Curve B, 1 γ, and Curve A, 0.5 γ, of desoxyribonuclease.

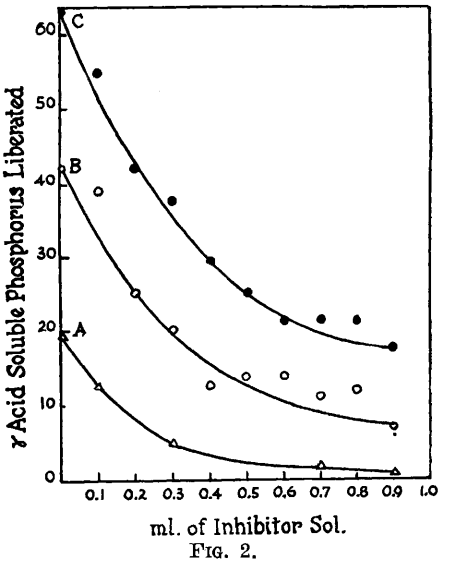

FIG. 2.

The effect of increasing amounts of inhibitor on the activity of desoxyribonuclease. Ordinate: gamma of acid-soluble phosphorus liberated, abscissa: ml of inhibitor solution added to the reaction mixture. The solution of inhibitor was the same as used in the experiments summarized in Fig. 1. Curve A, 0.5 γ; Curve B, 1.0 γ; Curve C, 1.5 γ of desoxyribonuclease.

1. Dabrowska, W., Cooper, E. J., and Laskowski, M., *J. Biol. Chem.*, 1949, v177, 991.

2. Hammarsten, E., *Biochem. Z.*, 1924, v144, 383.

3. Kunitz, M., *Science*, 1948, v108, 19.

4. Laskowski, M., and Saidel, M. K., *Arch. Biochem.*, 1945, v7, 465.

5. Kunitz, M., *J. Gen. Physiol.*, 1947, v30, 291.

TABLE I.
Distribution of Inhibitor in Pathological Human Tissues.

Case No.	Tissue	Viscosity units/g of fresh tissue
1	Breast adenocarcinoma	25.0
2	" "	8.3
3	Aberrant breast tissue (4 mo. pregnant)	0.8
4	Bowel adenocarcinoma	16.6
5	Gastric "	14.0
	Gastric mucosa (normal)	6.7
6	Sternal bone marrow (pernicious anemia)	11.7
	Sternal bone marrow (after Vit. B_{12})	10.0
7	Breast adenocarcinoma	2.0
	Lymph node (normal)	2.0
8	Cancer of breast	2.5
	Lymph node metastasis	1.8
9	Carcinoma of lymph nodes	1.5
	Lymph nodes (normal)	1.6

action between DRNase and the inhibitor is of the reversible type and probably obeys the law of mass action. An identical conclusion was reached from the experiment (Fig. 2) in which inhibition was determined by the acid soluble phosphorus method(6).

Table I shows the results of experiments in which human surgical material‡ was analysed for inhibitor. The content of inhibitor in malignant tissue varied widely from one case to another, while no striking differences were found between the malignant and non-malignant tissues of the same patient.

Regenerating rat livers were next investigated. Partial hepatectomy was performed§ according to Higgins and Anderson(7). No substantial difference in activity of DRNase between the regenerating and the control lobes could be demonstrated. This is in agreement with erratic changes reported by Novikoff and Potter(8) for desoxyribonucleic acid.

Finally several tissues of the normal rat were analysed for both DRNase and the inhibitor. The results of one of these experiments are shown in Table II. In several cases the same organ showed the presence of both enzyme and inhibitor simultaneously. This was most evident in the case of liver. Grinding with sand produced an extract rich in the inhibitor and comparatively poor in enzyme. A more efficient disintegration of the tissue in the Waring blendor resulted in an increased extraction of the enzyme and an apparent decrease of the inhibitor.

Table III shows the results of experiments in which normal rat livers were first "diced" by cutting with scissors, treated with 10 volumes of water, left to stand for 20 minutes at 5°, and centrifuged in the cold at 4,000 r.p.m. The supernatant was removed and analysed for both DRNase and the inhibitor. The tissue was then transferred to an ice-cooled Waring blendor and homogenized with an additional amount (10 volumes) of water. The homogenate was again centrifuged and the supernatant analyzed. The results indicate that the inhibitor is much more easily extractable than the enzyme. With the methods now available, it was not possible to determine the relative amounts of enzyme and inhibitor in the two fractions. It is obvious, however, that the inhibitor strongly predominated in the diced preparation, while the enzyme predominated in the blended.

In view of this finding further fractionation

TABLE II.
Distribution of Desoxyribonuclease and Its Inhibitor in Various Organs of the Rat.

Organ	Viscosity units of enzyme	Viscosity units of inhibitor
Liver (blendor)	5.8	.0
Liver (sand)	2.4	2.8
Kidney (blendor)	24.0	.0
Muscle (blendor)	1.3	.0
Lungs (blendor)	2.8	.8
Testes (blendor)	.0	1.0
Brain (sand)	.4	.0
Spleen (sand)	1.0	.4
Heart (sand)	3.3	.2

6. Laskowski, M., *Arch. Biochem.*, 1946, v11, 41.

‡ We are greatly indebted to Dr. M. M. Kaser, Dr. J. F. Kuzma, and Dr. M. Schuster for the samples of tissue.

§ We wish to express our appreciation to Mr. E. R. Haushalter for performing the operations.

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8. Novikoff, A. B., and Potter, V. R., *J. Biol. Chem.*, 1948, v173, 223.

TABLE III.
Effect of Homogenization on Activities of Extracts.

		"Diced"		"Blended"	
		DRNase (Viscosity units)	Inhibitor (Viscosity units)	DRNase (Viscosity units)	Inhibitor (Viscosity units)
Rat Liver	I	.0	.06	10.4	.0
	II	.0	.1	6.2	.024
	III	.0	.16	8.2	.0
	IV*	.0	.09	7.5	—
Bull Testes	I	.0	.1	1.0	.0

* Rat Liver III extracts (both diced and blended) were kept at 5° for 24 hours, and analyzed a second time.

TABLE IV.
Change in Activity of Extracts upon Standing at 5°.

	Fresh		2nd day		3rd day	
Tissue	DRNase	Inh.	DRNase	Inh.	DRNase	Inh.
Lymph node metastasis	.0	2.0	15	.0	.0	.0
" " (normal)	7.0	2.0	—	—	14.0	.0
Cancer of the breast	.0	1.8	7	.0	—	—

(Results are in viscosity units.)

of the liver into "soluble", "particulate", and "nuclei" fractions appeared advisable. This was accomplished using isotonic sucrose according to the method of Schneider(9). Neither the enzyme nor the inhibitor could be shown in the "nuclei" fraction. The "particulate" fraction always represented the fraction richest in enzyme, as would be expected from the previous observation. The "soluble" fraction was most erratic, showing the predominance of either enzyme or inhibitor. This was probably due to the fluctuations in the rate of liberation of desoxyribonuclease from the "particulate" fraction into solution.

Evidence that on standing the two components are destroyed at different rates, probably as a result of proteolysis, is presented in Table IV. The "blended' extracts prepared from human tissues, after being centrifuged, were exposed to 5° for different periods of time. The inhibitor was the first to disappear. The apparent increase in the amount of enzyme which was seen after 2 days' exposure was most probably due to the removal of the masking effect of the inhibitor, since on longer exposure desoxyribonuclease also disappeared. Thus far, attempts to devise a method which would quantitatively separate the enzyme and the inhibitor have proved unsuccessful.

Summary. The reaction between desoxyribonuclease and the inhibitor for desoxyribonuclease was found to be of the reversible type, similar to that observed by Kunitz between chymotrypsin α and soybean trypsin inhibitor.

A method for the semi-quantitative evaluation of the inhibitor for desoxyribonuclease has been described. Several normal and pathological tissues were analyzed for both desoxyribonuclease and the inhibitor. Both measurements were influenced by the presence of the second component of the pair.

The inhibitor was found to be readily soluble, while desoxyribonuclease was only slowly liberated into solution from the particulate matter of the cell. Neither of the two components was resistant to the proteolytic enzymes during autolysis, the inhibitor being the least stable of the two.

9. Schneider, W. C., *J. Biol. Chem.*, 1948, v176, 259.

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