TABLE II. Inhibition of Trypsin and Chymotrypsin by KNTI and PJTI.

Reaction mixture				
T, mg/ml	CH, mg/ml	KNTI, μg/ml	PJTI, mg/ml	Proteolytic activity
20				20
20		10		0
20			5	6
	20			20
	20	10		9
	20		10	20

T = Trypsin.
CH = Chymotrypsin.
KNTI = Kunitz-Northrop trypsin inhibitor.
PJTI = Pancreatic juice trypsin inhibitor.

preparing PJTI. This material inhibited trypsin (102 μg of trypsin inhibited/g wet weight of pancreas), was destroyed by incubation with trypsin or chymotrypsin, and failed to inhibit chymotrypsin. Thus, in regard to these properties, it was identical to PJTI. Similar extracts prepared from liver, small intestinal mucosa, muscle, and blood serum produced no inhibition of trypsin.

Discussion. In the original report on PJTI (1) the inhibitor could not be found in pancreatic juice which had been activated by enterokinase. This can now be explained by our finding that PJTI is susceptible to digestion by trypsin and chymotrypsin. Laskowski and associates(3,4) have systematically studied a variety of trypsin inhibitors including KNTI, another inhibitor prepared from pancretatic tissue by the method of Kazal *et al.*(5), blood plasma inhibitor, ovomucoid, soy bean inhibitor, and colostrum inhibitor. Only 2 of these inhibitors, Kazal's and ovomucoid, exhibited the properties described here for PJTI, namely inactivation by trypsin and failure to inhibit chymotrypsin. It is noteworthy that the inhibitor obtained from pancreatic tissue by simple extraction with trichloracetic acid has the properties of PJTI and not of KNTI, since both of these inhibitors are soluble in trichloracetic acid. On the basis of the properties studied to date Wazal's inhibitor and PJTI appear to be identical.

Summary. The trichloracetic acid filtrate of rat pancreatic juice contains an inhibitor of trypsin which does not inhibit chymotrypsin. The inhibitor is inactivated by trypsin and chymotrypsin.

This work was supported in part by grant from Nat. Science Fn. Mr. Ray Lichter gave expert technical assistance.

1. Kalser, M. H., Grossman, M. I., *Gastroenterology,* 1955, v29, 35.
2. Grossman, M. I., *Am. J. Physiol.,* 1958, v191, 535.
3. Laskowski, M., Wu, F. C., *J. Biol. Chem.,* 1953, v204, 797.
4. Wu, F. C., Laskowski, M., *ibid.,* 1955, v213, 609.
5. Kazal, L. A., Spicer, D. S., Brahinsky, R. A., *J. Am. Chem. Soc.,* 1948, v70, 3034.

Received July 21, 1958. P.S.E.B.M., 1958, v99.

Effect of Time of Injection on Removal of CA-45 and SR-85 by Peritoneal Lavage.* (24332)

ROY V. TALMAGE AND J. R. ELLIOTT†
Department of Biology, Rice Institute, Houston, Texas

Prior studies of ability of bone to supply calcium continuously to extra-osseous areas using the technic of peritoneal lavage, indicated that following parathyroidectomy in nephrectomized rats, amounts of calcium removed/hour fell to about 60% of that removed in parathyroid - intact controls(1). Additional studies(2) demonstrated that amounts of calcium removed could be increased significantly by addition of citrate to lavage rinse. In contrast, no significant changes in amounts of radiocalcium removed could be detected when radioactivity was injected 24 to 48 hours prior to start of lavage

* Aided in part by grant from Atomic Energy Comm.

† Present address: Department of Biochemistry, Jefferson Davis Hospital, Houston, Texas.

(3,4). These data were interpreted as indicating that parathyroid hormone and citric acid removed calcium from deeper areas of bone rather than from those portions in ready contact with extracellular fluid, whereas in the absence of parathyroids the major portion of calcium supplied by bone came from the more readily available surface areas of bone. The present studies were performed utilizing Sr^{85} in addition to Ca^{45}. Radiostrontium was used as indicator of calcium turnover in bone. Previous studies have shown only minor differences in removal of Sr^{85} and Ca^{45} from bone by peritoneal lavage(5). The primary difference was a slight preferential removal of strontium during continual lavage. Because of its different type of nuclear emission, radiostrontium provided a check against the more difficult radiotechnics in handling Ca^{45}. A 2-3 week delay between radioisotope injection and lavage procedure was used to allow radioactivity to become generally distributed throughout bone. If parathyroid hormone and administered citrate removed calcium from the deeper areas of bone, amounts of radioactivity removed by lavage should parallel total calcium removal.

Methods. Male Holtzman rats, weighing between 225 and 275 g were used; however, for any one experiment, the weight range was 25 g. Animals were on a calcium deficient diet for 3 days prior to use. Total nephrectomy was performed through a mid-ventral incision 24 hours prior to lavage. Parathyroids were removed individually at indicated time after start of continuous lavage. Operations were performed under nembutal or light ether anesthesia. As in previous studies describing lavage procedure in detail(1) "rinse" refers to fluid placed in the animal and "wash" refers to fluid collected from peritoneal cavity after stated equilibration periods. The peritoneal rinsing fluid was a calcium-phosphate free .85% NaCl solution containing 1.5% dextrose. Radiostrontium was administered in doses of 3-5 μc; radiomeasurements made with a NaI scintillation counter. Radiocalcium doses were 10-20 μc, and measurements made with a gas flow counter and corrected for self absorption. For comparative purposes, radioactivity removed during first lavage period was set at 1000 for each animal, and all other values for that animal adjusted relative to that value. Total calcium analyses were made with flame spectrophotometer as described previously(3).

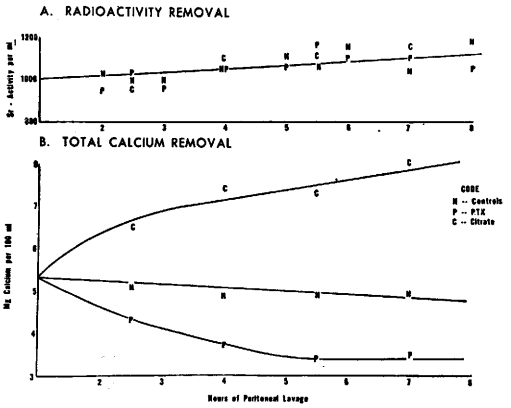

FIG. 1. Removal of Sr^{85} and stable calcium by peritoneal lavage. Sr^{85} inj. 24 hr prior to lavage. Animals parathyroidectomized during second wash, citrate added beginning with second wash. Avg stand. error $\left(\text{S.E.} = \pm \sqrt{\frac{d^2}{n(n-1)}} \right)$ of all points in 1A = 5% of values (max—10%). Avg stand. error of points in 1B = 3% of values (max—5%).

Results. Data from experiments involving removal of radiostrontium injected 24-48 hours prior to lavage are shown in Fig. 1. Neither parathyroidectomy nor administration of citrate affected the amount of radioactivity removed by lavage procedures (Fig. 1A). Total calcium removed, however, fell to about 60% of control values following parathyroidectomy. Addition of citrate to rinsing solution produced an opposite effect, increasing significantly the rate of calcium removal (Fig. 1B).

Results of experiments in which radioactivity was injected 2-3 weeks prior to lavage are shown in Fig. 2 and Table I. No differences in total calcium removal were seen, figures shown in Table I being similar to data in Fig. 1B. In contrast, considerable differences were seen in radiostrontium removal (Fig. 2) and radiocalcium removal (Table I). Here changes in rate of removal of radioactivity parallel total calcium removal seen in Fig. 1B.

Discussion. These studies extend previous work(2,3) and provide additional evidence

TABLE I. Removal of Ca^{45} Injected 2 to 3 Weeks Prior to Peritoneal Lavage.

	Exp. condition*	No. of animals	Lavage periods: 60′ washes 1	2	3	4	90′ washes 5	6
Radioactivity in c/m/ml	Neph	5	1000	914 ± 12	886 ± 31	887 ± 29	1034 ± 30	1045 ± 30
	PTX	4	"	937 ± 26	740 ± 42	721 ± 23	789 ± 27	844 ± 48
	Cit	4	"	925 ± 28	919 ± 19	1060 ± 27	1328 ± 40	1305 ± 44
Total calcium, mg Ca/100 ml	Neph	5	5.2 ± .27	5.0 ± .18	5.0 ± .14	5.0 ± .13	6.2 ± .27	6.3 ± .37
	PTX	4	5.2 ± .28	5.0 ± .16	3.8 ± .22	3.7 ± .07	4.6 ± .04	4.8 ± .09
	Cit	4	4.9 ± .21	4.9 ± .15	5.1 ± .39	6.3 ± .14	7.9 ± .46	7.5 ± .49

* PTX = parathyroidectomized during second lavage period. Cit = citric acid (25 mg %) wash started with second lavage period. Neph = nephrectomized only.

Radioactivity of 1st wash set at 1000, all other values given relative to first wash.

Values given with S.E. $= \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

that calcium is removed from bone by 2 processes. The basic process appears to be an equilibrium between the fluid media of body and the readily available calcium of bone (those areas which radiostrontium and radiocalcium are able to penetrate immediately after injection). This is shown by failure of parathyroidectomy or citrate administration to influence Sr^{85} removal rates when the isotope was injected shortly before lavage, even though total calcium removal was markedly altered. These data substantiate earlier studies (3,4) which used Ca^{45}.

The second process for calcium removal appears to involve action of parathyroid hormone on deeper areas of bone. Citric acid also appears to remove calcium from these areas. This is indicated by the parallel (but not necessarily equivalent) changes due to parathyroidectomy or citrate administration seen in removal of total calcium and both Sr^{85} and Ca^{45} when the activity was injected 2-3 weeks prior to lavage. These experiments also demonstrate the close relationship between citric acid and parathyroid hormone.

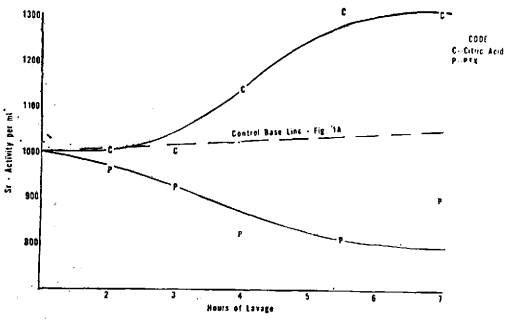

FIG. 2. Effect of time of inj. on Sr^{85} removal by peritoneal lavage. Sr^{85} inj. 3 wk prior to lavage. Animals parathyroidectomized during second wash, citrate added beginning with second wash. Avg stand. error of all points = 5% of values. The following comparisons are significant ($p < .01$): (1) Radioactivity removed after 3rd hr of lavage in animals parathyroidectomized *vs* those nephrectomized only. (2) Radioactivity removed after 4th hr of lavage in animals given citrate rinse *vs* those nephrectomized only.

Summary. 1) By use of peritoneal lavage, removal of Sr^{85} and Ca^{45} from bone has been studied relative to time of injection of radioactivity. Earlier studies had shown that parathyroidectomy or addition of citrate to lavage rinse had no effect on removal of radiocalcium injected 24 to 48 hours prior to start of lavage. In these experiments, similar results with radiostrontium substantiated earlier studies. In addition, experiments in which radioactivity, both calcium and strontium, remained in the animal 2 to 3 weeks before lavage procedures were done. In these experiments, parathyroidectomy reduced amount of radioactivity removed, while addition of citrate to the rinse increased it. Under these situations, therefore, changes in rate of removal of radioactivity paralleled changes in removal of total calcium. 2) These experiments are additional evidence that calcium is removed from bone by 2 processes, one possibly an equilibration phenomenon between fluid media of body and those portions of bone with which it is in closest contact. The second is removal of calcium from deeper areas of bone, under the influence of hormone of the parathyroids.

1. Talmage, Roy V., Elliott, J. R., *Endocrinology*, 1957, v61, 256.

2. Elliott, J. R., Talmage, Roy V., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 596.

3. Talmage, Roy V., Elliott, J., *Endocrinology*, 1958, v62, 717.

4. Elliott, J. R., Talmage, Roy V., *ibid.*, 1958, v62, 709.

5. Talmage, Roy V., Schooley, J. C., Comar, C. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 413.

Received July 21, 1958. P.S.E.B.M., 1958, v99.

Ultra-Violet Motion Picture Studies of Effects of Ribonuclease and Deoxyribonuclease on Living HeLa Cells.* (24333)

P. O'B. MONTGOMERY AND W. A. BONNER

Dept. of Pathology, University of Texas Southwestern Medical School, Dallas, Texas

The technic of time lapse motion picture photography by means of the ultra-violet flying spot television microscope has been previously described by the authors(1-4). With this technic it is possible to obtain time lapse motion pictures of undamaged living cells as they appear when illuminated with ultra-violet light at 2600 A. The motion pictures thus obtained represent principally the nucleic acid absorption images of the living cells. The typical appearance of the undamaged intermitotic HeLa cell obtained with this technic has been reported(2,4). It seemed of some interest to utilize this technic to record the effect of ribonuclease and deoxyribonuclease on U.V. absorption images of living HeLa cells, and this communication reports the results of this study.

Methods. The time lapse ultra-violet motion pictures were obtained by recording on one film frame the ultra-violet absorption image obtained by exposing the living cell to one complete one second scan of ultra-violet emitting cathode ray scanner tube employed as an illuminating source in this technic. During the following one second scan the scanner tube raster was electronically blanked so that no emission of ultra-violet occurred. This one second interval was utilized for the purpose of pulling the next camera film frame into position. The ultra-violet absorption image of the living cell produced by the following one second sweep of the ultra-violet scanner tube was then recorded on the new film frame following which the entire cycle was repeated. In this way the living cells and the film frame were only exposed to the scanning spot of ultra-violet light on alternate one second sweeps. HeLa cells were studied. They were prepared on quartz flying cover slip preparations in a media consisting of Hank's balanced salt solution, human ascitic fluid and embryo extract. The cultures were studied on either second or third day after sub-culture and all cultures contained vigorous healthy cells when examined by phase microscopy. These cover slip preparations were then prepared for study by mounting them on quartz slides in Hank's balanced salt solution containing ribonuclease or deoxyribonuclease. Three concentrations of ribonuclease were used, ½ mg/ml, 1 mg/ml, and 2 mg/ml. Two concentrations of deoxyribonuclease were used, 1 mg/ml and 4 mg/ml. The quartz slide cover slip preparations were then placed in the ultra-violet flying spot television microscope where they were kept at 37½°C and observed and photographed continuously for 6 hours. Replicate cultures were placed in the identical ribonuclease and deoxyribonuclease solutions and in Hank's balanced salt solution alone and were similarly incubated at 37½°C. Samples of each of these latter cultures were then removed at 1, 2, 4 and 6 hours and fixed in osmic acid and formalin. Following this fixation they were stained with Methyl-green-Pryonin, with Azure Blue B, and with the Fuelgen stain.

Results. When living HeLa cells were incubated in a solution containing ½ mg of

* This work was supported by Damon Runyon Memorial Fund and Southwestern Medical Fn.