TABLE III. Fatty Acid Composition of Serum Sphingomyelin.

Acid	Subject 1	2	3	4	5	6	7	8	9	10	Avg	Pooled serum*
14:0	1.0	.8	1.1	.6	1.0	.9	.6	.8	.3	.9	.8	.7
15:0	tr	.2	.4	tr	.3	.1	.1	.2	.2	tr	.2	.2
16:0	41.0	35.6	39.6	58.7	48.7	64.0	28.4	60.1	56.2	55.8	48.8	41.7
17:0	tr	.2	.4	tr	.7	.2	.2	.2	.2	tr	.2	.6
18:1	.9	1.2	2.4	1.6	3.7	3.4	1.2	1.7	2.9	.8	2.0	.7
18:0	7.3	9.2	16.7	15.9	7.7	6.8	5.6	5.5	8.1	19.5	10.2	9.4
19:0	.9	.3	.9	.7	.1	.7	.8	1.5	1.4	tr	.7	.3
20:0	1.8	1.9	2.4	2.1	2.8	1.6	1.8	1.2	1.4	1.2	1.8	3.9
21:0	.6	.3	.4	tr	.3	tr	.5	.4	1.0	.9	.4	.3
22:1	.5	tr	tr	.2	tr	1.5	tr	tr	tr	.6	.3	.4
22:0	7.4	9.5	9.2	5.3	10.0	4.4	12.3	4.6	5.9	2.9	7.2	12.1
23:1	tr	tr	tr	.4	.3	tr	tr	tr	tr	2.6	.3	.5
23:0	5.6	6.3	4.9	2.6	4.1	1.4	9.9	5.2	3.5	7.3	5.1	5.1
24:1	23.0	22.1	12.2	7.7	10.8	11.2	21.6	12.2	8.6	5.2	13.5	14.1
24:0	10.0	12.5	9.0	3.9	8.5	3.0	17.0	6.4	10.3	2.3	8.3	8.9
25:0	tr	tr	tr	tr	1.1	.8	tr	tr	tr	tr	.2	.4

* Pooled human serum analyzed by Sweeley(5).

and unsaturated fatty acids while sphingomyelin contains a series of saturated and monounsaturated fatty acids ranging from 14 to 25 carbon atoms. The major fatty acids of lecithin were 16:0, 18:0, 18:1 and 18:2 while the major sphingomyelin acids were 16:0, 18:0, 22:0, 24:1 and 24:0. There were no apparent age-dependent changes in the fatty acid composition of these lipids (16-56 years). The fatty acids of sphingomyelin appeared to vary in composition from subject to subject more than those of lecithin.

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Effects of Trypsin and Chymotrypsin on Blood Glucose *in vivo* and Glucose Uptake *in vitro*.* (30322)

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Rieser and Rieser(1) have described an *in vitro* insulin-like activity of several proteases and have reported that trypsin and chymotrypsin are particularly active in promoting glycogen synthesis and the accumulation of 3-0-methyl glucose. In addition they have reported that insulin has proteolytic properties(2). It seemed reasonable that if the insulin-like *in vitro* effects of these pro-

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teases had any physiological significance, the enzymes should produce hypoglycemia *in vivo*. This paper reports the effect of these enzymes on the blood glucose of dogs and rats and the effect of the enzymes on the glucose uptake of rat hemidiaphragms and epididymal fat pads *in vitro*.

Methods. For the *in vivo* experiments male Holtzman rats weighing 150-180 g were fasted for 24 hours and anesthetized with nembutal (30 mg/kg). Trypsin, chymotrypsin or insulin were given either intraperitoneally (I.P.) or intravenously (I.V.) via the femoral vein. Blood samples were taken from the tail for blood glucose determination.

Male dogs were anesthetized with nembutal (30 mg/kg) and an I.V. drip of the enzymes or insulin was started in the femoral vein. The rate of infusion for the enzymes on a molar basis was equivalent to 1 unit insulin/kg/hr. Blood samples for glucose determination were collected from the carotid artery.

For the *in vitro* experiments rats weighing 150-170 g were fasted for 24 hours prior to removal of hemidiaphragms and epididymal fat pads. The tissues were placed in flasks containing one ml of Krebs Ringer bicarbonate buffer with 2.50 mg of glucose. Each incubation was composed of a control group and 3 experimental groups containing equimolar concentrations of either trypsin, chymotrypsin or insulin. The flasks were incubated in a Dubnoff metabolic shaker at 37°C in a gas phase of 95% O_2 - 5% CO_2. The incubation period for the hemidiaphragms was one hour, and for the fat pads it was 3 hours. At the end of this time glucose disappearance from the medium was determined by the method of Somogyi(3) and expressed as mg glucose/g tissue/incubation period. The statistical significance of the difference between means was determined by the t test(4).

Results. Neither trypsin nor chymotrypsin produced an appreciable reduction in blood glucose of the rat as may be seen in Table 1. The transient hypoglycemia seen in 2 animals given chymotrypsin was insignificant when compared to the effect of equivalent amounts of insulin (compare rats 4 and 5 to rat 10).

Table II presents data collected on 3 dogs given trypsin, chymotrypsin, and insulin at the rate of 7.34×10^{-3} mM/kg/hr (equivalent of 1 unit insulin/kg/hr). The enzymes and insulin were dissolved in 0.9% NaCl and administered by I.V. drip. Dog A weighed 16.8 kg and received a total of 11.3 mg of trypsin in 4½ hours. Dog B weighed 13.6 kg and received a total of 9.2 mg of chymotrypsin in 4½ hours. Dog C weighed 25 kg and received a total of 1 mg (25 units) of insulin in one hour. Neither enzyme produced

TABLE I. Effect of Trypsin, Chymotrypsin or Insulin on Blood Glucose of Rats.

Rat	Quantity compound, mM × 10^{-3}/rat†	Blood sugar, mg % Initial	30 min	60 min	90 min
	Trypsin				
1	14.70*	67	70	70	74
2	3.67	66	66	66	74
3	3.67	66	90	90	90
	Chymotrypsin				
4	14.70*	78	55	82	74
5	14.70*	78	59	82	94
6	14.70*	74	82	101	140
7	3.67	66	82	98	74
8	5.87	98	82	82	98
9	7.34	66	66	82	109
	Insulin				
10	3.67	78	23	39	39
11	5.87	78	47	19.5	19.5
12	7.34	70	39	39	31
13	14.70	104	56	44	28
14	14.70	104	66	44	24

* Injected I.P. All other rats were injected I.V.
† 3.67 equivalent to .5 u insulin.
5.87 " " .8 u " .
7.34 " " 1.0 u " .
14.70 " " 2.0 u " .

TABLE II. Effect of Trypsin, Chymotrypsin or Insulin on Blood Glucose of Dogs.

Time of sample after start of infusion	Dog A*	Dog B*	Dog C*
	Blood sugar, mg %		
0	84	104	128
30	92	104	72
60	92	102	44
90	84	104	48†
120	84	90	50
150	90	90	
180	90	92	
210	90	78	
240	94	84	
270	94†	80†	
300	92	84	
330	94	90	

* Dog A received trypsin; Dog B received chymotrypsin; Dog C received insulin.
† Infusion stopped.

TABLE III. Effect of Trypsin, Chymotrypsin or Insulin on Glucose Uptake of Hemidiaphragms and Epididymal Fat Pads.

Sample	Hemidiaphragms	Fat pads
	Glucose disappearance	
Control	4.78 ± .25 (10)	1.51 ± .15 (10)
Insulin*	5.81 ± .24 (10)§	6.18 ± .54 (9) §
Trypsin*	4.96 ± .37 (10)	2.83 ± .47 (9)
Chymotrypsin*	5.03 ± .30 (10)	1.28 ± .29 (9)
Control	4.58 ± .40 (5)	2.33 ± .19 (5)
Insulin†	6.94 ± .14 (4) §	6.28 ± .46 (5) §
Trypsin†	4.85 ± .47 (4)	2.66 ± .42 (5)
Chymotrypsin†	4.49 ± .31 (5)	2.40 ± .29 (5)
Control	3.39 ± .35 (10)	1.28 ± .18 (10)
Insulin‡	5.65 ± .44 (10)§	5.65 ± .48 (10)§
Trypsin‡	3.93 ± .29 (10)	.66 ± .13 (10)§
Chymotrypsin‡	3.89 ± .20 (10)	.52 ± .10 (9) §

* .1 u/ml or 7.34×10^{-4} mM/ml. † .2 u/ml or 1.47×10^{-3} mM/ml. ‡ .5 u/ml or 3.67×10^{-3} mM/ml. § Where indicated, $P < .01$ for groups compared to control. All unmarked values not significant.

hypoglycemia in contradistinction to the effect of insulin which produced an effect in the first 30 minutes.

Failure to demonstrate an *in vivo* effect of these enzymes on blood glucose prompted an investigation of the *in vitro* effect of the enzymes on glucose uptake of hemidiaphragms and fat pads. Table III presents data collected when rat hemidiaphragms and fat pads were incubated in Krebs Ringer bicarbonate buffer with 2.50 mg glucose/ml. The tissues were incubated with insulin, trypsin, or chymotrypsin present in molar concentrations equivalent to 0.1 μ insulin/ml, 0.2 μ insulin/ml, and 0.5 μ insulin/ml. Insulin in these concentrations gives a significant increase in glucose uptake; however, neither enzyme produced an increase with the exception of trypsin at a concentration of 7.34×10^{-4} mM (0.1 μ insulin/ml). Both enzymes produced a very significant inhibition in the fat pads at a concentration of 3.67×10^{-3} mM (0.5 μ insulin/ml). The medium after the incubation of the fat pads with trypsin had a very milky appearance suggesting destruction of the tissue.

Discussion. Rieser and Rieser(1) using intact hemidiaphragms have shown that trypsin and chymotrypsin produced an insulin-like effect on the uptake of xylose and 3-0-methyl glucose. If the enzymes were used at a molar concentration equivalent to 0.5 μ insulin/ml, the effect of chymotrypsin on xylose was greater than that of insulin, and trypsin particularly promoted glycogen formation when either sugar was used. The authors point out that the opportunity for a histidyl-serine interaction (the active site of trypsin and chymotrypsin) is present in the insulin molecule.

Our failure to find a lowering of blood sugar in either dogs or rats using these enzymes warranted an investigation of the *in vitro* effect of trypsin and chymotrypsin on glucose in both hemidiaphragms and fat pads. It was felt that if insulin's active center was the same as that of trypsin and chymotrypsin, the enzymes should promote glucose uptake not only at a molar concentration equivalent to 0.5 μ insulin/ml but also at concentrations equivalent to 0.1 μ/ml and 0.2 μ/ml.

As the data show the enzymes were without any stimulatory effect on glucose uptake except for the slight but significant effect of trypsin on the fat pad at 7.34×10^{-4} mM (0.1 μ insulin/ml). Both enzymes produced a significant inhibition of glucose uptake at the highest concentration used presumably by their proteolytic effects.

These studies do not rule out the fact that the enzymes hold some properties in common with insulin; however, it would appear that physiologically these compounds are not acting in the same manner. The failure to show an *in vitro* action of the enzymes toward glucose considering the effect of these enzymes on uptake of xylose and 3-0-methyl glucose and formation of glycogen is not clear.

Summary. Experiments have been undertaken to extend the observation of Rieser and Rieser that chymotrypsin and trypsin have insulin-like activity *in vitro*(1) and that insulin has some protease properties(2). Concentrations of trypsin and chymotrypsin equal to molar concentrations of insulin which cause a marked hypoglycemia *in vivo* were administered to rats and dogs. Neither trypsin nor chymotrypsin had any insulin-like activity *in vivo*. These enzymes were also tested on the *in vitro* glucose uptake of rat hemidiaphragms and epididymal fat pads.

Only trypsin had a marginal effect on glucose uptake, an effect that was much less than the effect of an equimolar concentration of insulin.

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A Defect in Cellular Immunity During the Incubation Period of Passage A Leukemia in C3H Mice.* (30323)

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A previous report has documented the reduced antibody-forming capacity to T_2 bacteriophage in C3H mice incubating Gross passage A leukemia(1). This study was undertaken to define further the extent of the immunologic deficiency subsequent to infection with this leukemogenic virus, by assaying the ability of mice infected at birth with this agent and destined to become leukemic at a later age to develop the capacity to reject allografts. Since the reduction in antibody-forming capacity was found to be quantitative rather than qualitative, we decided to graft across a relatively weak (non H-2) histocompatibility barrier.

Materials and methods. Animals. C3H/Bi strain mice obtained from the original colony of Dr. J. J. Bittner were used. They were weaned and grafted at 4 weeks of age. All mice were housed individually following grafting. Ce/J strain mice obtained from R. B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used as donors. Donors were of the same age and sex as recipients.

Passage A virus. Leukemic C3H mice, representing the 32nd passage of Gross' passage A leukemia were obtained from Dr. Ludwik Gross in June, 1962. The virus was passaged in C3H/Bi mice as reported by Gross(2) until used in the 42nd passage for the present experiment. The experimental mice were given 0.2 ml of the cell-free filtrate intraperitoneally on the third day of life.

Grafting technique. When the mice were 4 weeks of age, they received a 1.5 cm^2 graft onto the mid-dorsal area. Two such grafts were obtained from the same region of each donor mouse. After removal of the underlying fascia the grafts were turned 90° and were sutured in place with 16 interrupted 6-0 ophthalmic silk sutures. Recipient mice were anesthetized with nembutal given by the intraperitoneal route.

Grafts were inspected 3 times a week until rejection occurred or until the animals were about to expire from leukemia. A graft was considered to be rejected when the donor skin had sloughed completely. Partial rejection was represented by gross shrinkage of the graft with only minimal hair growth on the remaining part of the graft. Acceptance of the graft in the controls was characterized by luxuriant hair growth. In the experimental animals 2 types of acceptance were seen: a) full acceptance with hair growth as in the controls and b) maintenance of the graft

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