TABLE I. Mean Change in Serum Electrolytes

		CO_2 (meq/L)	Cl (meq/L)	K (meq/L)
Tricosapeptide (deblocked)				
5 subjects	6.7 mg* peptide/d/6d	+1.1	+1.10	−.44
	6.7 mg peptide/d/7d	+ .9	−1.54	−.64
Tricosapeptide (blocked)				
4 subjects	6.4 mg peptide amide/d/6d	−1.2	+2.82	−.60
	6.4 mg peptide amide/d/7d	−1.3	+1.88	−.28

*equals 160 subcutaneous adrenal ascorbic acid depleting units.

insulin tolerance tests (0.1 unit of commercial regular insulin per kilo of body weight).

Results. There were no increases in blood pressure or body weight nor did any of the subjects develop Cushingoid manifestations. The deblocked tricosapeptide increased the urinary 11-oxysteroids and to a lesser extent the 17-ketosteroids while administration of the blocked congener, with the exception of one specimen in one patient, did not produce these changes. The sole exception occurred after the seventh injection of the blocked tricosapeptide (Figs. 2, 3).

The serum electrolytes showed a slight rise in CO_2 and a decrease in chloride and potassium when the deblocked tricosapeptide was administered, in keeping with changes induced by commercial ACTH. With the blocked tricosapeptide only the serum potassium values decreased (Table I).

The glucose and insulin tolerance tests were not altered by the blocked tricosapeptide whereas the deblocked polypeptide may have manifested a slight hyperglycemic and perhaps anti-insulin effect(6). Neither was there any pattern of change in serum lipids elicited by the blocked molecule.

Summary. Substitution of acetyl for hydrogen in the NH_2 group of the N-terminal serine, NH_2 for OH^- in the carboxyl of glutamine in position 5, and formyl for an amino hydrogen of lysine in positions 11, 15, 16, and 21 decreases or eliminates the ACTH-like activity of a synthetic tricosapeptide which duplicates the first 23 amino acids of naturally occurring ACTH.

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Repopulation of Thymus by Immunologically Competent Cells Derived from Donor Marrow. (26996)

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The cortical region of the thymus becomes atrophic following X-irradiation(1); however, the thymus recovers weight rapidly in irradiated mice that receive an injection of isologous(2,3) or parental bone marrow(2). This recovery may result from repopulation of the thymus by implanted cells, although Kaplan

* Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.

et al.(4) reported that 6 of 9 lymphomas developing in the thymuses of irradiated (C57BL × BALB/c) F_1 mice that had received parental bone marrow did not contain cells derived from the injected marrow. Moreover, the thymus did not return to normal weight in irradiated mice injected with homologous or heterologous bone marrow(2). Such observations suggest that the thymus may not be repopulated by parental, homologous, or heterologous marrow cells, although it has been shown that such marrow will repopulate other hematopoietic tissues of irradiated mice(5). On the other hand, Gengozian *et al.*(6) found that thymuses of lethally irradiated mice that received an injection of rat (heterologous) bone marrow did contain cells identified cytologically and serologically as rat cells.

Additional information as to the type of cells in the thymus of radiation chimeras seemed desirable in view of these apparent differences. Hence, an experiment was designed to determine the preponderant type of lymphopoietic cell (donor or recipient) in regenerated thymuses of irradiated F_1 hybrid mice given bone marrow cells of parental origin. The results of this study are reported herein.

Materials and methods. Four stocks of mice were used in this investigation: 1) C57BL/Cum, 2) 101/Cum, 3) (C57BL/Cum × 101/Cum) F_1, and 4) (C57BL/6 Cum × DBA/2 Cum) F_1. Stocks will be referred to as C57BL, 101, $B1F_1$, and $B6D2F_1$ mice, respectively. Age of the mice varied from 4 to 6 months. Approximately equal numbers of each sex were used, except that all of the $B6D2F_1$ mice were males.

The scheme of the experiment was as follows:

X-irradiated controls. $B1F_1$ and $B6D2F_1$ mice were given 650 r to determine the effect of X-rays† alone on their survival.

X-irradiated, thymus-injected mice. A single dose of 650 r, which was sublethal for $B1F_1$ mice and a LD 13/30 days dose for $B6D2F_1$ mice, was given 1 to 4 hours before such mice received an intraperitoneal injection of a thymus cell suspension. Thymus glands were excised from C57BL, 101, and $B1F_1$ donor mice. A cell suspension was made by cutting thymus glands of 5 donors into small pieces with scissors and by flushing the fragments several times through an 18- or 20-gauge hypodermic needle. The equivalent of one donor thymus was injected intraperitoneally into each of the 650 r X-irradiated recipients.

X-irradiated, regenerated thymus-injected mice. Thirty million bone marrow cells of strain C57BL or 101 mice were injected intravenously by tail vein into irradiated C57BL, 101, or $B1F_1$ mice within 4 hours after receiving a lethal dose of X-rays; a dose of 950 r was given to $B1F_1$ and 101 mice, and 850 r to C57BL mice. Thymus glands of such bone marrow recipients were excised 21 days later; thymus cell suspensions were prepared by the above method, and the cells were injected intraperitoneally into 650 r X-irradiated F_1 hybrid mice.

Thymuses of a few lethally irradiated, bone marrow-treated mice were fixed in Zenker-formol solution. Lymph nodes, spleen, and bone marrow of a few "X-irradiated, thymus-injected mice" and "X-irradiated, regenerated thymus-injected mice" were also fixed in Zenker-formol. Hematoxylin-eosin-stained sections of these tissues were prepared for histological examination.

Results. An X-ray dose of 650 r was sublethal for $B1F_1$ mice, and the survival of such mice was not altered by an injection of isologous thymus cells (Table I, lines 1 and 2). In contrast, 83% of 650 r-irradiated $B1F_1$ mice died within 90 days after receiving C57BL thymus cells and 50% died within 90 days after receiving 101 thymus cells (Table I, lines 3 and 4). Regenerated thymus tissue of lethally irradiated, isologous bone marrow-treated C57BL or 101 donors killed sublethally irradiated $B1F_1$ mice as, or more, effectively (Table I, lines 5 and 6) as the thymus cells of nontreated parental strain mice.

A second series of experiments was done to assay whether thymus cells in lethally irradiated $B1F_1$ mice that had received an injection of parental marrow cells were of recipients or donor origin.

For this series, a $B6D2F_1$ hybrid having only the parental donor strain in common with the $B1F_1$ bone marrow recipients was used. As

† Physical factors were: 300 kvP; 20 ma; 4.75 mm Be inherent filtration, 3.0 mm Al added filtration; 93.7 cm target-mouse distance; output ~90 r/min; HVL, 0.558 mm Cu.

TABLE I. Survival of Sublethally Irradiated F_1 Hybrid Mice Injected with Thymus Cells.

Donor Strain	Recipient Strain	No.	Survival (%) Days after treatment 10	30	60	90
1. —	$B1F_1$	48	100	100	100	100
2. $B1F_1$	idem	48	98	98	98	98
3. C57BL	"	48	85	48	25	17
4. 101	"	48	100	69	58	50
5. C57BL/C57BL*	"	30	93	37	23	17
6. 101/101*	"	64	94	36	23	9
7. —	$B6D2F_1$	52	100	87	85	83
8. $B1F_1$	idem	24	96	92	92	92
9. C57BL	"	24	96	0	0	0
10. $B1F_1$/C57BL†	"	56	100	5‡	0	0

*Isologous bone marrow chimeras (see text). †Parent-F_1 bone marrow chimeras (see text).
‡All 30-day survivors were dead 40 days after treatment.

indicated in Table I (lines 7 and 8), 650 r of X-rays was lethal for 17% of the $B6D2F_1$ hybrids, and an intraperitoneal injection of $B1F_1$ thymus cells did not further affect survival. However, $B6D2F_1$ mice given 650 r of X-rays and injected with C57BL thymus cells died within 30 days after treatment (Table I, line 9). Similarly, irradiated $B6D2F_1$ hybrids that received regenerated thymus cells from lethally irradiated C57BL marrow-treated $B1F_1$ donors died within 40 days after treatment (Table I, line 10). Although C57BL and C57BL/6 mice are of different strains, their major histocompatibility factors are similar, and C57BL thymus cells did induce a wasting disease in irradiated $B6D2F_1$ mice.

Pathological findings. Histological examination showed that much of the thymus cortex had regenerated within 21 days in $B1F_1$ mice given parental bone marrow. $B6D2F_1$ mice given regenerated thymus tissue from C57BL bone marrow-treated $B1F_1$ donors showed granulomatous reticular cell hyperplasia and hypertrophy in the lymph nodes, splenic white pulp, and Peyer's patches of the intestine. These active granulomatous foci were similar to those observed in sublethally irradiated $B1F_1$ mice injected with thymus cells from normal C57BL or 101 donors and in the irradiated $B6D2F_1$ mice injected with C57BL thymus cells. The histological findings are also similar to those occurring in sublethally irradiated F_1 mice injected with parental spleen cells (7) ‡.

Discussion. The conclusion drawn from this study is that the thymus in a lethally irradiated F_1 hybrid given parental marrow is repopulated 21 days after treatment by donor marrow cells. A more rapid recovery of thymus weight in irradiated F_1 hybrids receiving parental bone marrow than in those not receiving marrow (2) suggested that the thymus was actually repopulated, even though some of the thymic lymphomas that developed later in such mice were of host type (4). Our study was made using the newly regenerated thymus, whereas the thymic lymphomas tested by Kaplan *et al.* (4) appeared after a latent period of more than 80 days. The type of thymus cells in such late radiation chimeras may not be the same as 21 days after bone marrow transplantation since grafts of parental marrow may regress within 90 days or longer after implantation (8). The mortality in 650 r-irradiated F_1 mice injected with parental lymphoid cells is attributed to genetically determined histocompatibility differences between tissues of the recipient and donor; *i.e.*, immunologically competent cells of parental strain mice produce antibodies against tissue antigens of the F_1 hybrid recipient (9,10,11). Pathological findings support the view that the sublethally irradiated $B1F_1$ recipients of parental strain thymus cells died as a result of an immunological reaction, a conclusion reported previously by Kaplan and Rosston (12). Studies of Congdon and Duda (13) also suggest that thymus cells injected into irradiated mice may give rise to antibody and plasma cells.

Summary. Many of the sublethally irradiated $B1F_1$ and $B6D2F_1$ mice injected intraperitoneally with thymus cells from parental strain donors died within 90 days, owing presumably to an immunological reaction by the

‡ The author is grateful to Dr. G. E. Cosgrove for his interpretation of the histological preparations.

implanted cells against the recipient. Likewise, regenerated thymus tissue of lethally irradiated $B1F_1$ mice injected with C57BL bone marrow cells induced a fatal wasting disease on implantation in sublethally irradiated $B6D2F_1$ mice. It was concluded, therefore, that regenerated thymus tissue in lethally irradiated $B1F_1$ mice injected with C57BL bone marrow was repopulated by immunologically competent cells derived from the donor marrow.

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Effect of Ribonuclease Upon Immunologically Active Cells.* (26997)

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Brent *et al*(1) have demonstrated that lymphoid cells of homograft sensitized animals provoke a cutaneous reaction of the delayed type (the "transfer reaction") when injected intradermally in the antigen donor. The specificity of this reaction is consistent with 2 possibilities: that the transfer reaction is dependent on "cell-bound antibodies" or "endoantibodies" or that humoral antibodies are produced in such small quantities that their effect can be detected only in the immediate vicinity of the cells which produce them(2). The character of the reaction itself, which is an indurated inflammatory lesion, implies either that host cells infiltrate the injection site or that the transferred cells increase in mass by multiplication and/or synthesis of new cytoplasmic material. All these features require an intact protein synthesis.

In the present experiments we have applied a biochemical technique to this immunological model to evaluate the role of protein synthesis by immunologically active cells in producing the reaction. Ribonuclease provides a useful tool for investigating this problem. Allfrey *et al*(3) demonstrated in experiments with microsomes that the integrity of ribonucleic acid may be deleteriously affected by ribonuclease with a resulting inhibition of protein synthesis, and Brachet(4), and Ledoux and Baltus (5) have shown that ribonuclease penetrates many living cells and is able to slow protein anabolism without killing the target cells.

Materials and Methods. Dutch rabbits were immunized with slices of New Zealand rabbit ear skin implanted subcutaneously. Ten days later draining lymph nodes were recovered, minced in cold Hanks' solution and a cell suspension prepared. Active ribonuclease (5 × crystallized, Nutritional Biochemicals Corp.) or ribonuclease inactivated by heat (30 min at 100°C) were added to aliquots containing equal numbers of cells to give a final concen-

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