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CORTISONE RESISTANT THYMOCYTES

PHILIP LAWRENCE COHEN

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_5 May 1972_____

Date

CORTISONE RESISTANT THYMOCYTES

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of the

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in Partial Fulfillment of the

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SUMMARY

The DNA synthetic response to sheep erythrocytes and in a graft-versus-host reaction of adoptively transferred cortisone resistant thymocytes was measured in irradiated mice and compared to the response of thymocytes harvested from untreated donors.

It is generally believed that cortisone resistant thymocytes are the only immunocompetent cells in the thymus.

Results indicate that

1. Cortisone sensitive thymocytes are not inert and account for a significant portion of thymocyte response in a graft-versus-host reaction.

2. Cortisone resistant thymocytes respond to antigen by a markedly different pattern of DNA synthesis than untreated thymocytes, being chiefly a more labile population. This suggests that there exist interactions between cortisone-sensitive and cortisone resistant thymocyte populations.

3. Direct evidence is presented that cortisone resistant thymocytes may exert immunosuppressive effects on whole thymocyte populations.

4. A role is proposed for variations in the corticosteroid secretion rate, related to stress, as a possible nonspecific con-

trolling factor in the mediation of thymus-derived lymphocyte

responses to antigen.

I. INTRODUCTION

1. Corticosteroid-Lymphocyte Interactions

Interrelations between adrenal cortical steroids and the
immune system were first noted by Thomas Addison (1) who described
lymphoid hyperplasia in patients suffering from adrenal insuffi-
ciency. With the isolation of relatively pure adrenal cortical
preparations in the 1940's, it became apparent that lymphocytes,
and in particular thymic lymphocytes, were exquisitely sensitive
to small doses of corticosteroids (2, 3). In the thymus, cortico-
steroid administration is followed by marked edema and by exten-
sive dissolution of medium and small cortical lymphocytes (the
term lymphocytokarryorrhexis was coined by Dougherty to describe
the "shedding of cytoplasm" observed.) The cytocidal effect of
corticosteroids on the thymus was the basis of an early in vitro
bioassay for adrenal steroid activity (4).

Early experiments indicated an anatomically selective action
of corticosteroids on the thymus. Dougherty (2, 5) observed that
medullary reticulendothelial cells and a small population of large
medullary lymphocytes were relatively resistant to the action of
corticosteroids. Ishidate and Metcalf (6) showed evidence of

vigorously dividing cortisone resistant lymphocytes clustered around
PAS-positive reticulum cells. Esteban (7) demonstrated that short-
lived thymocytes were most susceptible to the destructive effects
of corticosteroids.

Attempts to elucidate corticosteroid mechanisms of action on
thymocytes have met with mixed success. On the basis of in vitro
experiments (8, 9, 10, 11) it appears that a hydroxyl-group on
the 11-beta position is essential for steroid action on thymocytes.
Cortisone, thus, must be converted to cortisol (hydrocortisone) by
reduction at the 11 position, a reduction readily achieved in vivo
by the liver (12).

17-HYDROXY-CORTICOSTERONE:
CORTISOL, "F"

11-DEHYDRO-17-HYDROXY-
CORTICOSTERONE : CORTISONE,
"E"
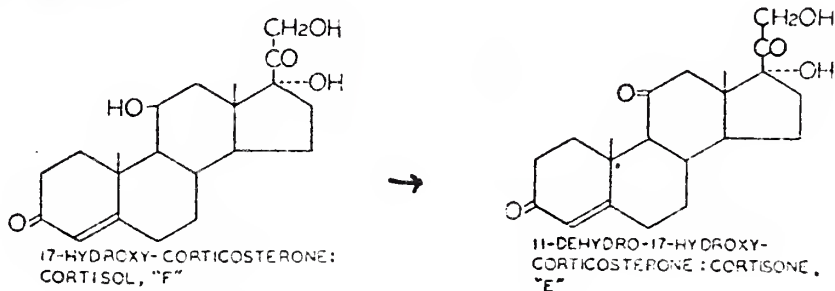
Major biochemical effects of cortisol on thymocytes are inhi-
bition of RNA, DNA, and protein synthesis and decreased transport
of nucleic acid and protein precursors into the cells (12 - 18).
Inhibition of DNA synthesis may in part be due to direct inhibition
of thymidine kinase (20). The relation of these biochemical phe-
nomena to lymphocytolysis is unclear (21).

Recent evidence suggests that steroid effects are mediated by corticosteroid binding to specific nuclear receptors (22). There appear to be about 5000 glucocorticoid binding sites, located in the nucleus (not on the nuclear membrane). A protein-steroid complex is rapidly formed after incubation of thymocytes with cortisol (23). There is little evidence for any action on cytoplasmic membranes.

A probable biochemical mechanism for the apparent selective steroid effect on cortical thymocytes may be extrapolated from the literature. Dougherty et al. (5) demonstrated that the ability to resist cortisol cytolysis depends on the ability of the lymphocyte to oxidize cortisol to biologically inactive cortisone, hence protecting itself from cytocidal effects. Though no direct proof has been published, it would seem plausible to assume that cortisone resistant thymocytes resemble peripheral lymph node lymphocytes in the presence of 11-beta-hydroxysteroid dehydrogenase on their cell membranes and are hence resistant to the action of cortisol.

It is interesting to speculate on the biological implications of steroid-lymphocyte interactions. Ambrose (24, 25) has documented an absolute requirement for cortisol in in vitro antibody-synthesizing organ culture systems. Under these conditions,

physiological levels of cortisol are essential, yet higher concentrations inhibit antibody synthesis. On the basis of elegant biochemical studies, the author concluded that the "permissive" effect of corticosteroid in the formation of antibody involved the derepression of genes by unmasking sites of the DNA of chromatin for attachment of natural signals or inducers. Whether this is a B or T cell effect is unknown.

It seems likely, however, that T-cells would be affected, in view of their known greater sensitivity to steroid effects (26, 27). The effects of stress, and its concomitant increased corticosteroid secretion rate, on the immune response may in part be due to genetic derepression events on regulatory T-cells (28).

Whatever the teleological reasons for steroid-lymphocyte interactions, it seems unlikely that the adaptations of lymphocytes to corticosteroids are purely accidental.

2. <u>Functions of Thymic and Thymus-Derived Lymphocytes</u>

In the past decade, it has become clear that the thymus is
fundamental to the immune response, both cellular and humoral.
Lymphocytes derived from the bone marrow enter the thymus; an
unknown proportion of these leave the thymus after an undetermined
time and are fundamentally changed as a result of their sojourn.
The present state of knowledge of thymus physiology has been
reviewed extensively elsewhere (29) and will be outlined only
briefly here.

Essentially, the thymus may be regarded as a producer of long-
lived "thymus-derived lymphocytes", or "T-cells." Far more is
known about the behavior of these cells than is known about the
processes involved in their production and differentiation, a
topic to be considered later. Thymus-derived lymphocytes are
relatively small (8 microns), have a large nuclear:cytoplasmic
ratio, and have been shown to be long-lived (30). In humans,
their life span ranges in excess of 15 - 20 years, and they have
been estimated to constitute 80% of peripheral blood lymphocytes
(31). Thymus-derived lymphocytes have the property of recirculating,
that is, they pass intracellularly from the blood through the pos-
capillary venules of lymph nodes, enter the lymph nodes and travel

through the lymphatic system, ultimately re-entering the blood through the major lymphatic ducts. Recirculation through the spleen has also been demonstrated.

"T-cells" are to be distinguished from short-lived "B-cells", or bone-marrow derived lymphocytes, which have not passed through the thymus and do not recirculate.

Thymus-derived lymphocytes carry on their surfaces a distinctive antigen, termed theta (θ), and tend to localize in specific "thymus dependent" areas of the lymphoid system, namely the paracortex of lymph nodes and the periarteriolar sheaths of the spleen (33). They are the prime participating cells in delayed hypersensitivity reactions (cellular immunity), and appear to be the source of the chemical mediators of these reactions.

It is also well documented that thymus-derived lymphocytes are capable of mounting graft-versus-host reactions (34, 35). They are also capable of blast transformation in response to phytohemagglutinin and other mitogens (36), and participate in mixed lymphocyte reactions (58). Chromosome labelling studies have shown them to undergo mitosis in response to antigen (37). This response can be reduced by pretreatment with the same antigen (38).

3. Thymus-Derived Lymphocytes as Interacting Cells

Thymus-derived lymphocytes are incapable of producing anti-
bodies when infused into lethally irradiated hosts (39, 40). A
major advance of the past few years, however, was the demonstration
by Claman and Chaperon in 1967 that thymus-derived lymphocytes
are capable of interacting synergistically with bone-marrow-derived
lymphocytes in the formation of antibody (30). The validity of
this finding is no longer in doubt, but is interpretation remains
a matter of conjecture. The mechanisms proposed for lymphocyte
cooperation have been reviewed by Miller et al. (40) and have
chiefly attributed antigen concentration as a property of T-cells,
antibody production as a property of B.-cells. T-cells, which
contain on their surfaces small amounts of immunoglobulin, are
thought either to focus antigen passively onto B-cells by
antigen-bridging; or perhaps to interact specifically with antigen,
differentiating, dividing, and producing a factor or factors
mediating the induction of the immune response. The putative
factor has been termed by some IgX, though there is no direct
evidence for its being an immunoglobulin.

Whatever the nature of T-cell interactions, an impressive
body of literature has arisen incriminating such interations in

various immunological phenomena. In addition to their by now well-documented role in cooperation with B-cells to form at least some, if not all antibodies, T-cells have been demonstrated to cooperate with bone marrow in the production of graft-versus-host spleno-megaly (41). Cantor and Asofsky (42) have reported synergistic interactions between thymic lymphocytes and splenic and lymph node lymphocytes in the production of graft-versus-host reactions; these results may be interpreted as evidence for the existence of T-cell interactions with other T-cells.

Recent evidence supports the concept of negative interactions among thymus-derived and other lymphocytes. Gershon and Kondo (42) demonstrated a key role for thymus-derived lymphocytes in the induction and breaking of tolerance to sheep erythrocytes in mice, and more recently have shown that T-cells from mice made unrespon-sive to sheep erythrocytes are capable of blocking the antibody response to this antigen even in fully reconstituted bone marrow chimera mice (44). Antigenic competition, the mechanism whereby administration of antigen leads to diminished response to subse-quent administration of unrelated antigen, has been shown to be a T-cell mediated phenomenon (45). Baker (46) presented further evidence for an immunosuppressive role for T-cells in experiments where thymus deprived mice were shown to produce excess antibody

to certain antigens; in these animals the addition of T-cells was shown to depress the excess antibody titres.

Herzenberg (47) presented an intriguing model of suppressive interactions between lymphoid cells to explain "allotypic suppression" in inbred mice. In this phenomenon, Fl mice, immunoglobulin allo-type a/b, are exposed in vitro or neonatally to anti-b antibody. They are subsequently unable to express the b immunoglobulin allo-type. This suppression seems to be due to supressive T-cells, lasts for years, and can be transmitted by adoptive transfer of allotypic lymphoid cells.

Gershon et al. (48) presented three different instances in which T-cells, in the absence of B-cells or antibody, could suppress the DNA synthetic response of other T-cells. "Educated" T-cells, harvested from spleens of antigen-challenged mice and adoptively transferred to irradiated recipients, were demonstrated to reduce the DNA synthetic response of "naive", unchallenged T-cells to sheep erythrocytes; Fl T-cells were observed to decrease the mitotic graft-versus-host response of parental T-cells administered to irradiated Fl mice; and cortisone-resistant T-cells were shown to cause a much reduced DNA synthetic response to sheep erythrocytes when combined with thymocytes harvested from untreated donors and adoptively transferred into lethally irradiated hosts.

Allison (49, 50) has attempted to unite under one formulation evidence for both negative and positive T-cell interactions. Whatever the mechanism turns out to be, it seems clear that T-cells play a fundamental regulatory role in immunity and are capable of initiating and promoting the immune response as well as being able to slow down or stop it. Liebhaber and Gershon (manuscript in preparation) have accumulated evidence for a bidirectional regulatory role of T-cells. In experiments on the kinetics of T-cells in graft-versus-host reactions, it was observed that, depending on the relative numbers of cells involved, Fl T-cells could exert either stimulatory or inhibitory effects on a graft-versus-host reaction initiated by infusing parental thymocytes into irradiated Fl mice.

4. <u>Heterogeneity of Lymphocytes within the Thymus</u>

In the current view, the thymus is thought of as a compartment in which lymphocytes become unalterably changed before release into the circulation as small, recirculating, lymph-node seeking "T-cells", paramount in cell mediated immunity and of great importance in many, if not all humoral immune reactions. Understandably, much interest has centered around the nature of the intrathymic lymphocyte "education" process.

It is known that thymic lymphocytes are extraordinarily active mitotically (51). The fate of the large volume of lymphocyte traffic in the thymus is not completely understood, but it is probable that lymphocytes enter the thymic cortex from the bone marrow, progressively decrease in size, migrate to the medulla of the thymus, and thence leave via the thymic vein to enter the paracortexes of peripheral lymph nodes and the periarteriolar sheaths of the spleen (52 - 55).

Though intrathymic lymphocytes are immunologically weak in comparison with lymph node and spleen lymphocyte populations (known to be rich in T-cells), nevertheless thymic lymphocytes possess definite immunologic competence in graft-versus-host reactions, in cooperation with B-cells to form antibody, and as

measured by mitotic response to allogenic cells and to mitogens
(56).

With the realization that thymic lymphocytes possessed a
degree of immunologic competence, evidence began to appear that
such competence was not uniformly distributed among thymocytes.

Warner (57), in 1964, showed that medullary cells of the
chick thymus, selected by their resistance to cortisone, were a
far more immunologically competent population than thymic cortical
cells, as judged by their ability to injure chick chorioallantoic
membrane. Weber (58), in 1966, showed that phytohemagglutinin
responding cells in the pig thymus were cortisone resistant and
localized in the thymus medulla, and in 1970 demonstrated that
these medullary thymocytes were able to participate in mixed
lymphocyte reactions almost as well as splenic lymphocytes; in
contrast, cortical thymocytes were said to show negligible partici-
pation (59).

Blomgren and Andersson (56), in 1969, showed that cortisone
resistant thymocytes were far more active, per cell, in inducing
graft-versus-host splenomegaly than were suspensions of thymocytes
derived from untreated mice. The interpretation that the cortisone
resistant thymocytes represented a population similar to peripheral
lymph node lymphocytes was supported by the observation that the

volume distribution of these two groups of cells was identical.

Cohen, Fischbach, and Claman (26) confirmed that cortisone resistant thymocytes were highly active in a graft-versus-host system and also found that cortisone resistant spleen and marrow lymphocytes were more active than comparable untreated cells in initiating graft-versus-host splenomegaly.

Andersson and Blomgren (60) showed in 1970 that the cortisone resistant fraction accounted almost entirely for the capacity of thymocytes to cooperate with bone marrow in the restoration of the humoral antibody response to sheep erythrocytes, bovine serum albumin, ovalbumin, and NIP determinant in lethally irradiated, thymectomized mice. Similarly, Cohen and Claman (61) observed that cortisone resistant thymocytes were highly active in interacting with bone marrow to form anti-sheep erythrocyte Jerne plaques in the spleens of bone marrow chimera mice. Further, the proliferation of thymus cells, once infused into recipients, was found to be uninfluenced by hydrocortisone (62).

Blomgren (63), in 1971, studied volume changes occurring when allogeneic thymocytes were infused into lethally irradiated mice and concluded that cortisone resistant medullary thymocytes were the sole thymocyte sub-population undergoing blastoid transformation in response to H-2 antigens. Blomgren and Andersson

(64), in 1971, confirmed the idea that medullary cortisone resistant thymocytes were highly effective in producing graft-versus-host reactions and in restoration of antibody production in bone marrow chimeras; they further concluded from these studies that cortical, cortisone sensitive thymocytes were inert in both of these immuno-logical phenomena.

Cortisone resistant mouse thymocytes were found by Blomgren and Svedmer, in 1971, to be highly responsive in vitro to phytohemagglutinin and highly reactive in a mixed lymphocyte reaction, confirming Weber's earlier similar experiments with pig tissues; furthermore, cortisone resistant thymocytes were said to be transformed into specific "killer cells", toxic to allogeneic fibroblasts, when cultured with allogeneic mitomycin-treated cells with the same major histocompatibility antigens (65).

Schimpl and Wecker, in 1971, deprived mouse spleen cell suspensions of their T-cells by treatment with anti-θ serum and complement; such suspensions were unable to respond significantly to sheep erythrocytes. Whereas ordinary thymocytes failed to restore the hemolysin response, thymocytes from hydrocortisone-treated mice were able to reconstitute the system fully (66).

Bach and Dardenne, in 1971, found that cortisone treatment of mouse thymuses augmented the percentage of spontaneous rosette

forming cells but that the actual number per thymus of rosette forming cells was unchanged (67).

In chromosome labelled thymus graft studies, Elliot et al., in 1971, presented direct evidence that the small phytohemagglutinin-responsive cell population in the thymus, while probably cortisone sensitive itself, is derived from the cortical cortisone sensitive population (68).

The theta (θ) surface antigen, mentioned above, has been shown to be a marked of thymus-derived lymphocytes in mice (69). Thymic lymphocytes have high θ-titres; and bone marrow-derived lymphocytes have zero titre. Cortisone resistant thymocytes were found by Raff in 1971 to have θ titres similar to those of peripheral T-cells and in Cr^{51} labelling studies were observed to have the same migratory pattern as peripheral thymus-derived lymphocytes (70).

The TL (thymus leukemia) antigen, like θ, has served as a useful marker in identifying thymic lymphocytes. In TL+ strains of mice, most thymocytes have high TL titres but the antigen is absent from extra-thymic lymphocytes (70). Schlesinger and Golakai, in 1967, presented good evidence that the small proportion of TL- thymocytes represented the same population as cortisone resistant thymocytes (71). Leckband and Boyse, in 1971, demon-

strated that the graft-versus-host potential of thymocytes is almost totally confined to TL- cells (72). Raff, in 1971, found that TL- thymocytes, like low-titre θ+ thymocytes, migrated to spleen and lymph nodes in a pattern indistinguishable from peripheral thymus-derived lymphocytes (70).

Colley, Shih-Wu, and Waksman have recently fractionated thymocyte populations by differential flotation in bovine serum albumin (73, 74). It appeared from these experiments that most of the immunocompetent thymic cells were located in three of five discrete bands, termed A, B, and C. However, these putatively immunocompetent thymocytes lacked certain characteristics of peripheral lymphoid cells, and possessed a different distribution.

A large body of evidence exists, then, suggesting that a small population of medullary thymocytes, which are cortisone resistant, TL-, low grade θ+, recirculating, and constitute perhaps 3-5% of the total cells in the thymus (56) -- are a highly active immunocompetent cell population within the thymus. These findings, however, have been interpreted as meaning that cortisone resistant thymocytes are the only immunocompetent cells in the thymus (56, 60, 61, 63 - 65). In this thesis, data is presented to the contrary, and its is proposed that there may be several interacting cell populations of immunological importance within the thymus.

II. EXPERIMENTAL

1. Experimental Plan

In a system free of B-cells and antibody, the DNA synthetic
response of cortisone resistant and other T-cells to sheep erythro-
cytes and in a graft-versus-host reaction was studied. Attempts
were made to demonstrate cellular interactions between cortisone
resistant thymocytes and other thymocytes.

2. Materials and Methods

Mice: Male C3H and C3D2F1 (C3H X DBA2) were obtained from
Jackson Laboratory, Bar Harbor, Maine. Mice were rested
in the animal facility for one week before use. Thymocyte
donors were 5 weeks old, recipients were 8 weeks old.

Irradiation: A Siemens Stabilipan 250 KV X-Ray machine,
with 2 mm. Al filter, was used for irradiation of recipients.
They were placed in a plexiglass chamber on a rotating plat-
form and received 900 R at a dose rate of 85 R per minute.
In experiments involving in vitro irradiated cell suspen-
sions, these were contained in non-leaded glass tubes, kept
ice-cold, and were slowly rotated while receiving a total of
900 R of X-irradiation at a rate of 85 R per minute, using
the Siemens 250 KV machine with 2 mm. Al filter.

Sheep Erythrocytes: These were maintained in refrigerated Alsever's solution and washed three times before use. They were inoculated intraperitoneally into recipient mice in a volume of 0.2 ml.

Cell Suspensions: Donor mice were killed by cervical dislocation and thymuses carefully dissected out under sterile conditions. Thymuses were then gently squeezed between two sterile glass slides. Cell suspensions thus obtained were then filtered through three layers of gauze and washed twice in ice-cold sterile medium 199 containing 100 units/ml. of penicillin, streptomycin and kanamycin and 10 units/ml. heparin. Viable cell counts were made with a hemocytometer using the trypan blue dye exclusion method (4).

Cortisone: Donor mice were given IP injections of 2.5 mg. cortisone acetate ("Cortone", Merck, Sharpe, and Dome). These and untreated animals were sacrificed 48 hours later and cell suspensions were then made from their thymuses.

Cell Inoculations: These were given intravenously via tail vein. The inoculated volume never exceeded 0.2 ml.

Technique for Assaying DNA Synthesis of Thymocytes: This
technique is described in detail by Gershon and Hencin (75).
Lethally irradiated mice were inoculated with the appropriate
experimental suspension of thymocytes on Day 0 of the experi-
ment. On the day of assay of DNA synthesis, mice were given
1×10^{-7} moles of 5-fluoro-2-deoxyuridine I.P. This com-
pound inhibits the formation of endogenous thymidine from
deoxyuridine monophosphate (76). One hour later these mice
received an I.P. injection of two microcuries of 5-iodo-2-
deoxyuridine labelled with ^{125}I (^{125}IUDR). (Amersham/
Searle, specific activity 4 - 6 microcuries per microgram).
The ^{125}IUDR is incorporated into DNA in the place of thymidine
monophosphate (76, 77). Twenty-four hours later the animals
were killed and the spleens and in some cases femoral lymph
nodes were dissected out and placed in counting tubes con-
taining formalin. At the termination of the experiment, the
spleens and nodes were counted for six minutes in a Nuclear-
Chicago scintillation counter. Also counted at this time
was a standard aliquot of 0.2 ml. ^{125}IUDR from the same lot
used in inoculating the test animals. The counts in the
experimental tubes were divided by the counts of the standard,
and the result was expressed as percent uptake of isotope.

All counts were corrected for background. Results were
expressed as uptake on a given day (e.g., Day 2). This
refers to the day of pulse injection of the ^{125}IUDR, i.e.,
in this case two days after inoculation with thymocytes.
One day later the animal was killed and its spleen removed.

Interpretation of Data

Where appropriate, results were compared using Student's
t-test.

III. RESULTS

These experiments were performed with several objectives in mind. The first was to observe the behavior of cortisone resistant thymocytes, compared to that of unfractionated thymocyte populations, in a relatively new assay system free of B-cells and antibody. The aim was to test the widely held notion that cortisone resistant thymocytes represent the sole immunocompetent intrathymic cell population. This was investigated both in response to sheep erythrocytes and in a graft-versus-host reaction. In addition, repeated attempts were made to document interactions between cortisone resistant thymocytes and whole thymocyte suspensions, and also between these cells after in vitro lethal irradiation.

1. DNA Synthetic Response to Sheep Erythrocytes

A. Response to a Single Challenge with Sheep Erythrocytes:

In several experiments, lethally irradiated C3D2F1 mice received intravenous infusions of syngeneic thymocytes and were challenged with one dose of sheep erythrocytes (0.2 ml. of a 10% suspension injected intraperitoneally) immediately thereafter.

The animals received an intraperitoneal injection of 10^{-7} moles of 5-fluoro-2-deoxyuridine on the day of assay, followed one hour later by an IP pulse of two microcuries of ^{125}IUDR. Twenty-four hours later, these mice were sacrificed by cervical dislocation and their spleens dissected out. The spleens were counted in a scintillation counter at the conclusion of the experiment and the percent uptake of infused radioisotope was calculated as described in the "Methods" section. Percent uptake is expressed as uptake on the day of pulse infusion of ^{125}IUDR.

A consistent finding in several experiments was the much higher isotope uptake, per cell, of cortisone resistant thymocytes compared to cells derived from untreated donors. Based on uptake per cell of radioisotope, cortisone resistant thymocytes were calculated to account for 80 - 90% of the total DNA synthetic response of whole thymocyte suspensions to sheep erythrocytes as measured in this assay system. In other words, the cortisone resistant thymocytes could almost on their own account for the entire response of unselected thymocytes to sheep erythrocytes (Figure 1 and Table 1).

The kinetics of isotope uptake of cortisone resistant thymocytes, i.e., the effect of varying the numbers of cells infused, was not unlike that observed for whole thymocyte suspensions (75).

TABLE 1 - Splenic uptake of ^{125}IUDR with multiple doses of antigen

No. Thymocytes	Cortisone Treated	SRBC Administration	% Uptake Per Cell	% Isotope Uptake ± SD	Day of Exp.
1.3 X 10^8	no	Day 0	1.01X10^{-10}	0.0131± 0.0012	2
			3.42	0.0445± 0.0094	3
			1.76	0.0229± 0.0147	4
	Overall Uptake		6.19	0.0805	2, 3, 4
2.4 X 10^6	yes	Day 0	4.62X10^{-9}	0.0111± 0.0031	2
			14.2	0.0340± 0.0054	3
			7.41	0.0178± 0.0067	4
	Overall Uptake		26.1	0.0629	2, 3, 4
1.3 X 10^8	no	Days 0, 1	0.638X10^{-10}	0.0038± 0.0030	2
			3.10	0.0404± 0.0174	3
			1.75	0.0228± 0.0131	4
	Overall Uptake		5.50	0.0715	2, 3, 4

TABLE 1 (Continued)

No. Thymocytes	Cortisone Treated	% Isotope Uptake ± SD	% Uptake Per Cell	SRBC Administration	Day of Exp.
2.4×10^6	yes	0.0104 ± 0.0050	4.33×10^{-9}	Days 0, 1	2
		0.0417 ± 0.0211	17.3		3
		0.0329 ± 0.0126	13.7		4
	Overall Uptake	0.0715	5.50		2, 3, 4
1.3×10^8	no	0.0235 ± 0.0078	1.80×10^{-10}	Days 0, 1, 2	3
		0.0162 ± 0.0061	1.24		4
	Overall Uptake	0.0397	3.05		3, 4
2.4×10^6	yes	0.0436 ± 0.0084	18.2×10^{-9}	Days 0, 1, 2	3
		0.0261 ± 0.0150	10.9		4
	Overall Uptake	0.0697	29.0		3, 4

FIGURE 1: Ratios of isotope uptake, per cell, of cortisone re-
sistant thymocytes: normal thymocytes, in response to multiple
challenges with sheep erythrocytes (see text).
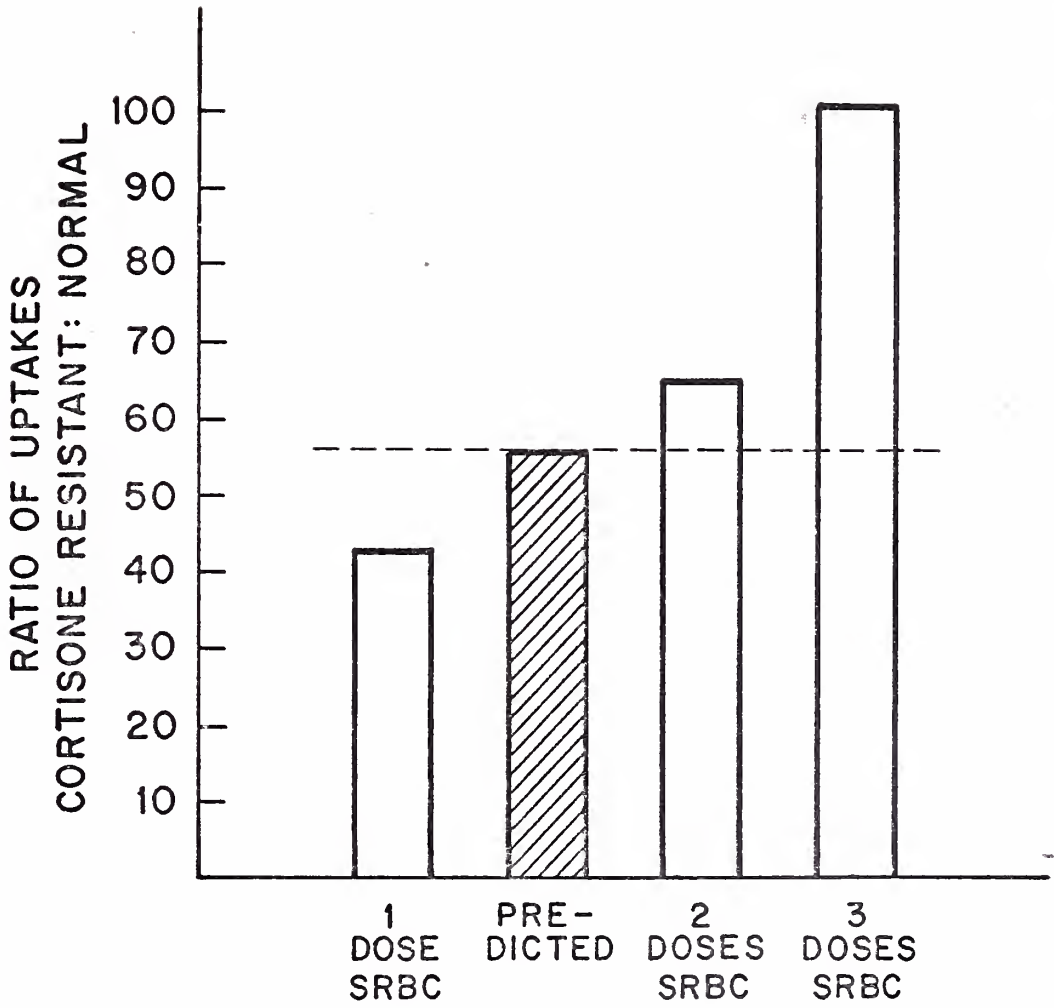
Specifically, a tenfold increase in the number of cortisone

resistant thymocytes was seen in another experiment to increase

the isotope uptake by a factor less than ten (Table 2). This

finding is not in apparent agreement with the in vitro experiments

of Andersson and Svedmer (65), who reported that cortisone resistant

thymocytes exhibited greater than first order kinetics, i.e., a

tenfold increase in cell number resulted in a greater than tenfold

increase in fibroblast killing activity.

B. Response to Multiple Challenges with Sheep Erythrocytes

The responses of cortisone resistant and normal thymocytes

to multiple I.P. challenges with 0.2 ml. of a 10% sheep erythro-

cyte suspension were compared, using the same experimental system

described in the previous section. In this experiment, untreated

donor thymuses yielded 54 times as many cells as cortisone treated

thymuses. If the cortisone resistant thymocyte population were

the sole immunocompetent intrathymic population, one would expect

that these cells would cause about 54 times as much splenic isotope

uptake per inoculated cell. Indeed, when one dose of antigen was

administered, the cortisone resistant cell response came close

to this expectation (Figure 1). With multiple challenges of anti-

gen, however, the relative potency of the cortisone resistant cells

TABLE 2

Number of Cortisone Resistant Cells Infused	Total ^{125}IUDR Uptake, Minus Control, $\pm$ SD
2×10^5	$0.015\% \pm 0.008$
2×10^6	$0.075\% \pm 0.005$

appeared to be almost twice as much as would be predicted on the basis of their relative numbers.

In contrast to the response of normal thymocytes, then, the response to antigen of cortisone resistant thymocytes was highly "boostable" -- these cells were able to increase their response greatly when challenged with repeated doses of antigen. Consequently, the ratio of their response to the relatively unchanged response of untreated cells was a function of antigen dose (Table 1 and Figure 1).

C. Evidence for Cellular Interactions

In a number of experiments, groups of animals received mixtures of cortisone resistant and normal thymocytes in attempts to document interactions between different thymocyte populations. In one experiment (Figure 2) a dramatic negative interaction was observed. The addition of $2X10^5$ cortisone resistant thymocytes to $2X10^7$ normal cells resulted in a combined response to packed SRBC much less than the response of $2X10^7$ normal cells alone. (p<.05). This phenomenon occurred on the day of peak response.

Repeated attempts to confirm this result were suggestive but inconclusive. No evidence was found for synergistic interactions between cortisone resistant and normal thymocytes. Figure

FIGURE 2: DNA synthesis produced by immunization with 0.2 ml. of
packed sheep erythrocytes of syngeneic thymocyte populations (see
(text).

              I - is the peak response of each group, independent
                   of which day it occurred.

             II - is the total response, measured daily, subdivided
                   into the daily response.

I. PEAK RESPONSE

II. TOTAL RESPONSE

% UPTAKE OF I^{125}UDR MINUS CONTROL

Days
4
3
2

A
Cortisone
Rx (2 x 10^5)

B
Normal
(2 x 10^7)

A+B

A
Cortisone
Rx (2 x 10^5)

B
Normal
(2 x 10^7)

A+B
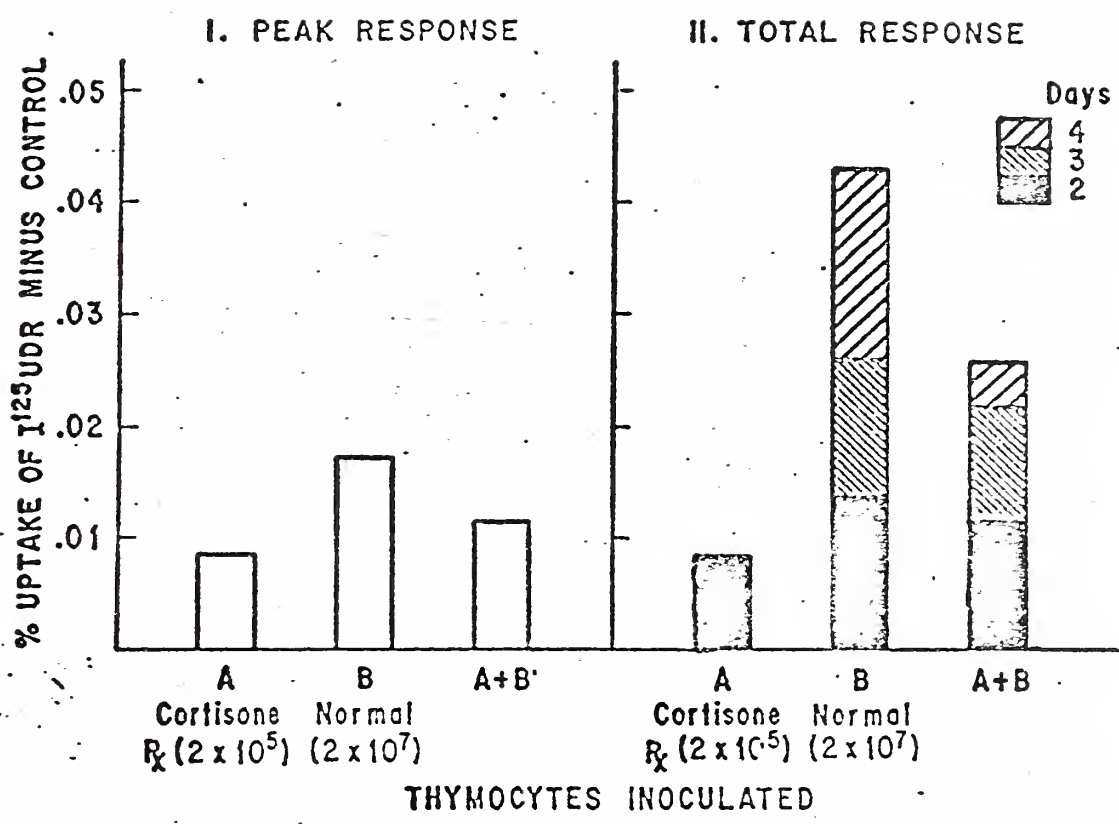
THYMOCYTES INOCULATED

3 shows the results of a representative experiment in which addi-
tion of variable numbers of cortisone resistant thymocytes to a
constant inoculum of untreated thymocytes resulted in a stepwise
increase in isotope uptake response to SRBC, fully explainable
by addition of cell responses and not suggestive of synergy.

Experience in our laboratory with a graft-versus-host system
(Liebhaber and Gershon, to be published) suggested a possible
suppressive role for irradiated thymocytes in our assay system.
Consequently, a pilot experiment was designed to determine
whether in vitro lethally irradiated (900 R) thymocytes were
capable of suppressing the response of whole and cortisone treated
thymocytes to sheep erythrocytes. The results (Figure 4) sug-
gested that lethally irradiated whole thymocyte suspensions might
depress the response of both cortisone resistant and untreated
thymocytes on the second day after challenge (p<.025) but this
was the only day tested and these interactions remain to be fully
explored.

2. Behavior of Cortisone Resistant Thymocytes in Graft-Versus-
    Host Reactions

A series of experiments was undertaken to explore the be-
havior of cortisone resistant cells in graft-versus-host reactions
involving infusions of C3H allogeneic thymocytes into C3D2F1 re-

FIGURE 3: Splenic uptake of ^{125}IUDR by various combinations of normal and cortisone treated thymocytes. Antigen was 0.2 ml. packed SRBC.

●————●   1 X 10^7 normal thymocytes

+––––––+   1 X 10^7 normal thymocytes + 2 X 10^5 cortisone resistant thymocytes

○—·—·—·—○   1 X 10^7 normal thymocytes + 2 X 10^6 cortisone resistant thymocytes

×··········×   2 X 10^5 cortisone resistant thymocytes

△— — — —△   2 X 10^6 cortisone resistant thymocytes
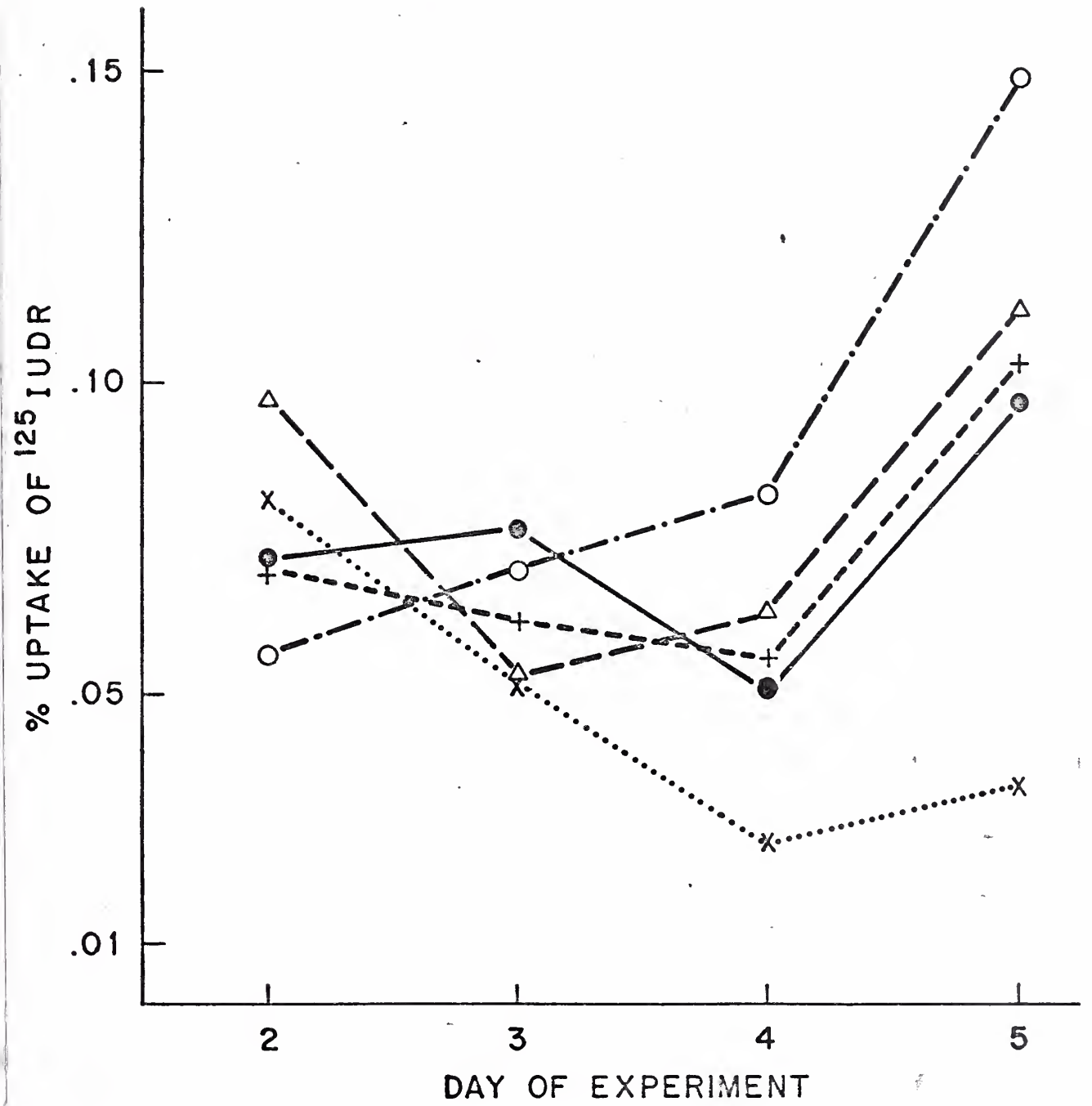
FIGURE 4: Percent splenic uptake of ^{125}IUDR in lethally irradiated mice given combinations of normal + irradiated thymocytes and cortisone resistant + irradiated thymocytes.

A: 5×10^7 normal thymocytes

B: 5×10^7 normal thymocytes + 5×10^7 lethally irradiated thymocytes

C: 1×10^6 cortisone resistant thymocytes

D: 1×10^6 cortisone resistant thymocytes + 5×10^7 lethally irradiated thymocytes
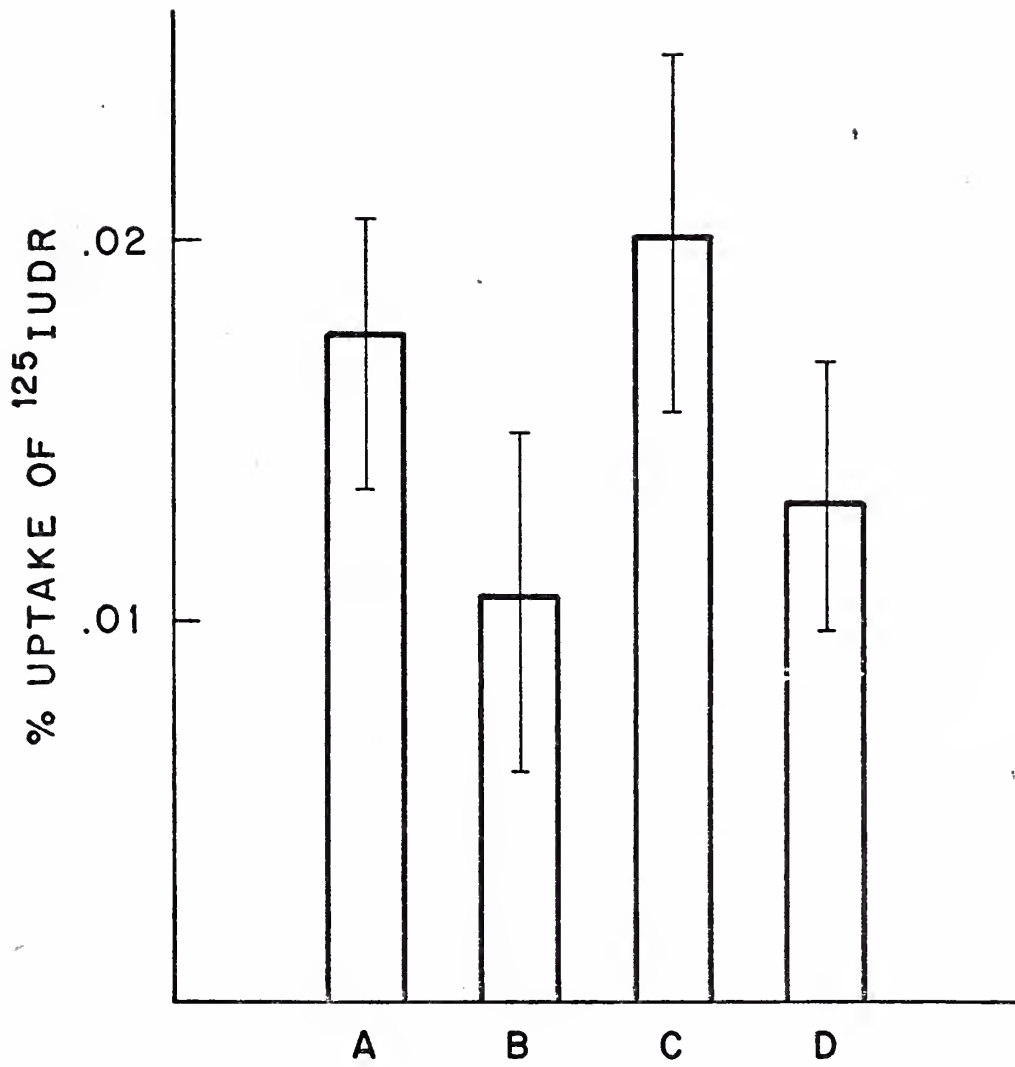
cipients. These two inbred strains of mice differ at the H-2
locus and it is known that a vigorous graft-versus-host response
occurs when the parental (C3H) thymocytes are infused into the
F1 (41).

In a representative experiment, seventeen times as many
thymocytes were recovered from untreated donor mice compared to
cortisone treated mice. The cumulative ratios of per cell splenic
and lymph node ^{125}IUDR uptake, however, were only about half the
ratios anticipated if these cortisone resistant cells accounted
for all the immunocompetent cells participating in the graft-versus-
host reaction. The relatively poor performance of cortisone
resistant cells in graft-versus-host reactions has been a constant
finding in several experiments (Figure 5 and Table 3).

A. Spleen and Lymph Node Isotope Uptake in Graft-Versus-Host
Reactions

In most graft-versus-host experiments, both spleens and
femoral lymph nodes were harvested and counted. Lymph node
results seemed to parallel spleen results. There was some indi-
cation that cortisone resistant thymocytes preferentially responded
in lymph nodes (Table 5).

TABLE 3 - Splenic uptake of ^{125}IUDR in a graft-versus-host reaction

No. Thymocytes	Cortisone Treated	% Splenic Isotope Uptake ± S.D.	% Splenic Uptake Per Cell	Day of Experiment
2×10^7	no	0.1099± 0.0033	5.49×10^{-9}	2
		0.1782± 0.0192	8.91	3
		0.1301± 0.0844	6.50	4
	Overall Uptake	0.4182	20.9	2, 3, 4
1.2×10^6	yes	0.0234± 0.0111	1.95×10^{-8}	2
		0.0843± 0.0208	7.02	3
		0.1015± 0.0076	8.45	4
	Overall Uptake	0.2092	16.4	2, 3, 4

TABLE 4 - Femoral lymph node uptake of ^{125}IUDR in a graft-versus-host reaction

No. Thymocytes	Cortisone Treated	% Node Isotope Uptake ± S.D.	% Node Uptake Per Cell	Day of Experiment
2×10^7	no	0.00484± 0.00137	2.42×10^{-10}	2
		0.02081± 0.00322	10.4	3
		0.01693± 0.01035	8.46	4
	Overall Uptake	0.04258	21.3	2, 3, 4
1.2×10^6	yes	0.00146± 0.00316	1.21×10^{-9}	2
		0.00690± 0.00254	5.75	3
		0.01258± 0.00562	10.4	4
	Overall Uptake	0.02094	17.3	2, 3, 4

FIGURE 5: Ratios of isotope uptake, per cell, of cortisone re-
sistant thymocytes:normal thymocytes, in a graft-versus-host
reaction (see text).

TABLE 5 - Ratios of splenic isotope uptake: lymph node isotope uptake
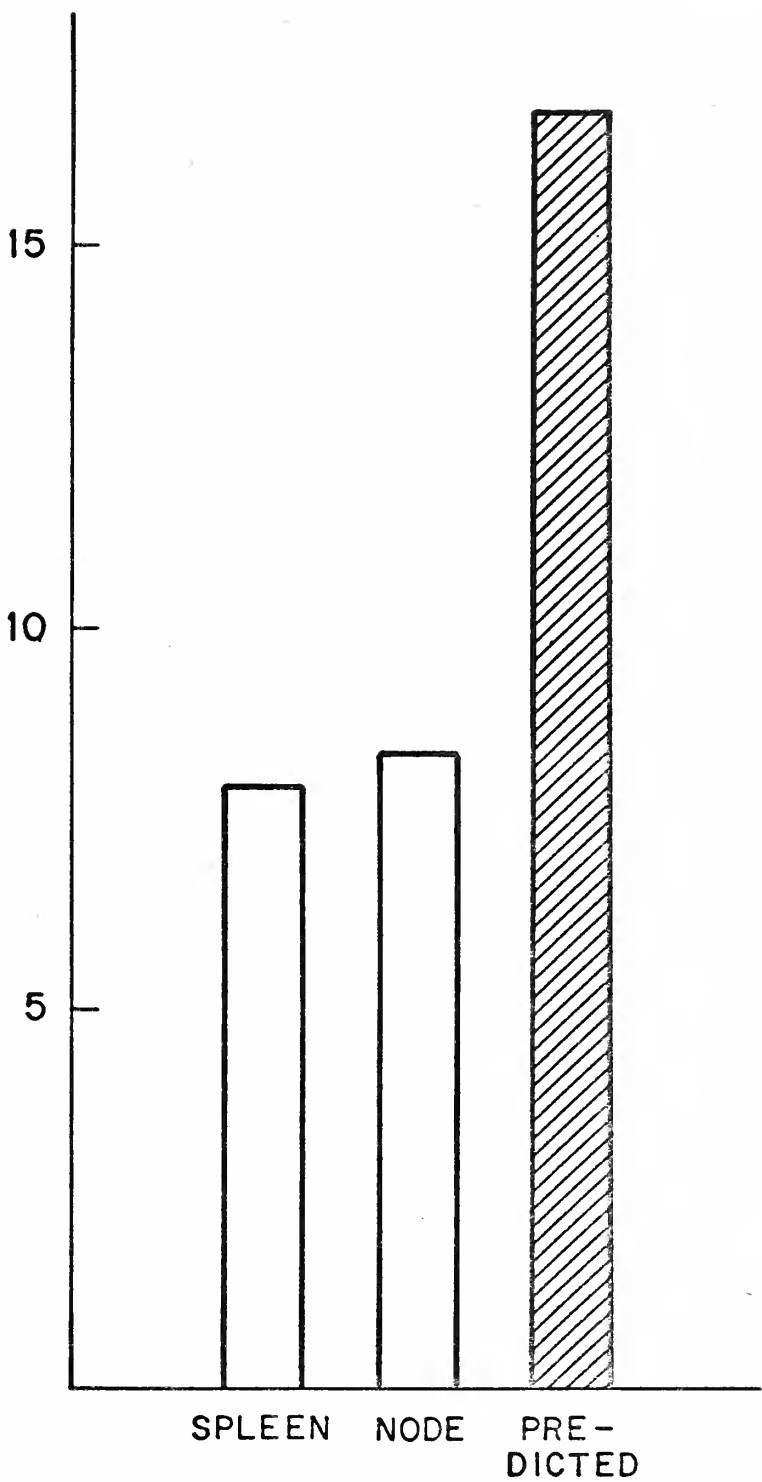in the graft-versus-host reactions described in Tables 8 and 9

Thymocyte Inoculum	Day of Experiment	Spleen:Node Uptake Ratio
4.5×10^7 normal	2	12.9
"	3	18.0
"	4	11.4
	Total (2, 3, 4)	14.9
3.15×10^6 cortisone resistant	2	9.7
"	3	11.7
"	4	7.5
	Total (2, 3, 4)	9.7

B. Cellular Interactions in Graft-Versus-Host Reactions

Several experiments were performed to explore possibilities
of cell interactions in graft-versus-host reactions. Previous
experiments (48) had indicated that Fl cells might have suppres-
sive effects on graft-versus-host reactions in Fl animals. Sup-
pression was often enhanced by administering 900 R of X-irradia-
tion in vitro to the Fl thymocytes before combining them with
parental cells.

Consequently, experiments were designed to elicit inter-
actions between cortisone resistant, irradiated, and untreated
thymocytes in graft-versus-host reactions.

Marked suppressive interactions ("antergy") was observed
when 6×10^6 cortisone resistant Fl thymocytes were added to 4×10^7
parental thymocytes and the combination infused into Fl mice in
the same experimental system described above. The combined response
was significantly (p<.05) less than the response of the parental
cells alone, both in spleen and lymph nodes. When the cortisone
resistant cells were exposed to 900 R of X-irradiation before
combination with the parental cells, an even greater suppression
(p<.005) occurred (Figure 6). As noted in sheep erythrocyte ex-
periments, optimum suppressive effect occurred late in the response,
in this case, one day after peak response.

FIGURES 6 and 7: Effect of combining _in vitro_ irradiated thymo-
cytes with normal thymocytes on a graft-versus-host reaction (see
text).

•————————• 4 X 10^7 normal C3H thymocytes

o— · —— · —o 4 X 10^7 normal C3H thymocytes + 6 X 10^6 cortisone
               resistant C3D2Fl thymocytes

X· · · · · · · · · ·X 4 X 10^7 normal C3H thymocytes + 5 X 10^5 _in vitro_
               irradiated cortisoen resistant thymocytes

+— — — — —+ 4 X 10^7 normal C3H thymocytes + 6 X 10^6 _in vitro_
               irradiated cortisone resistant thymocytes

SPLEENS

LYMPH NODES
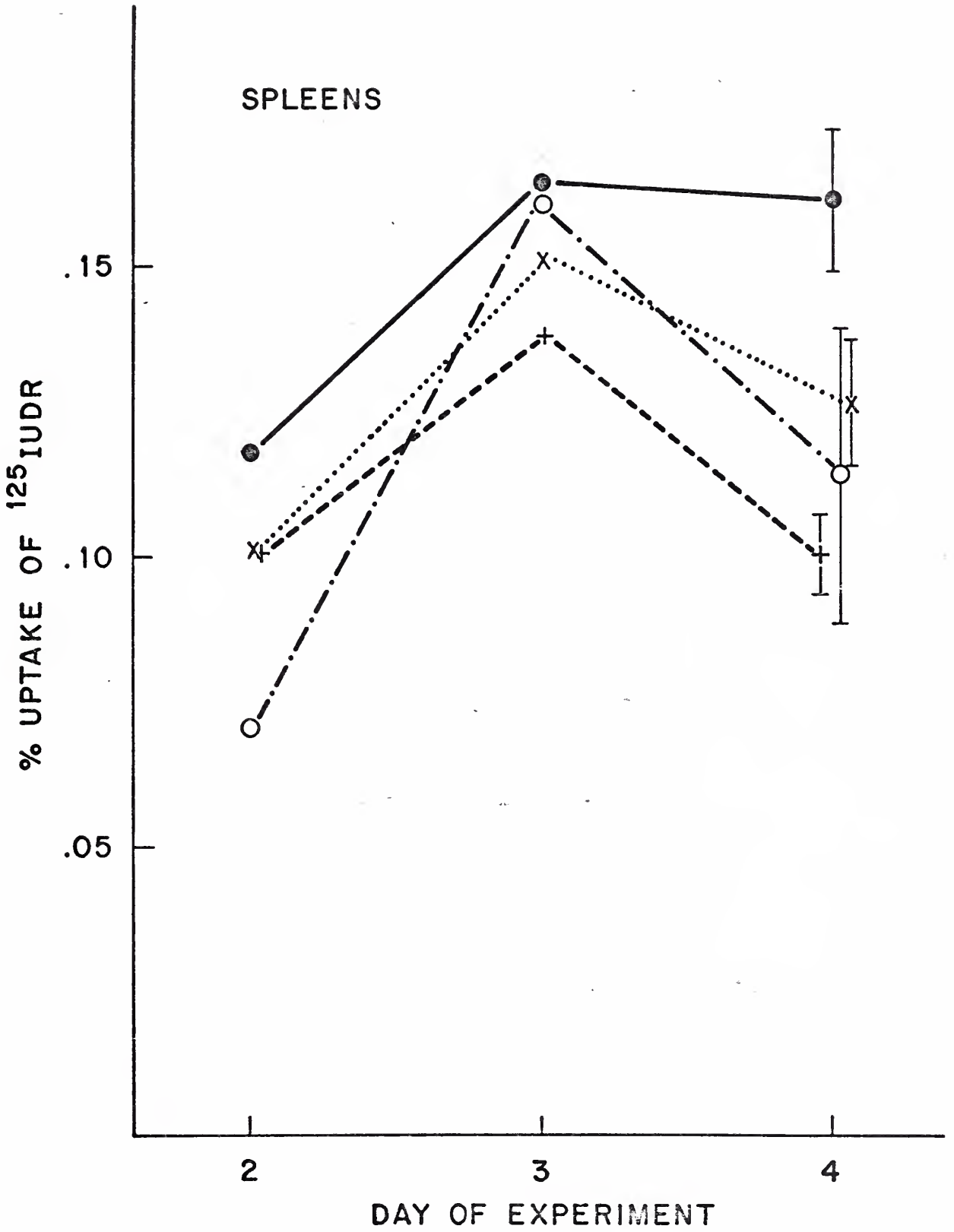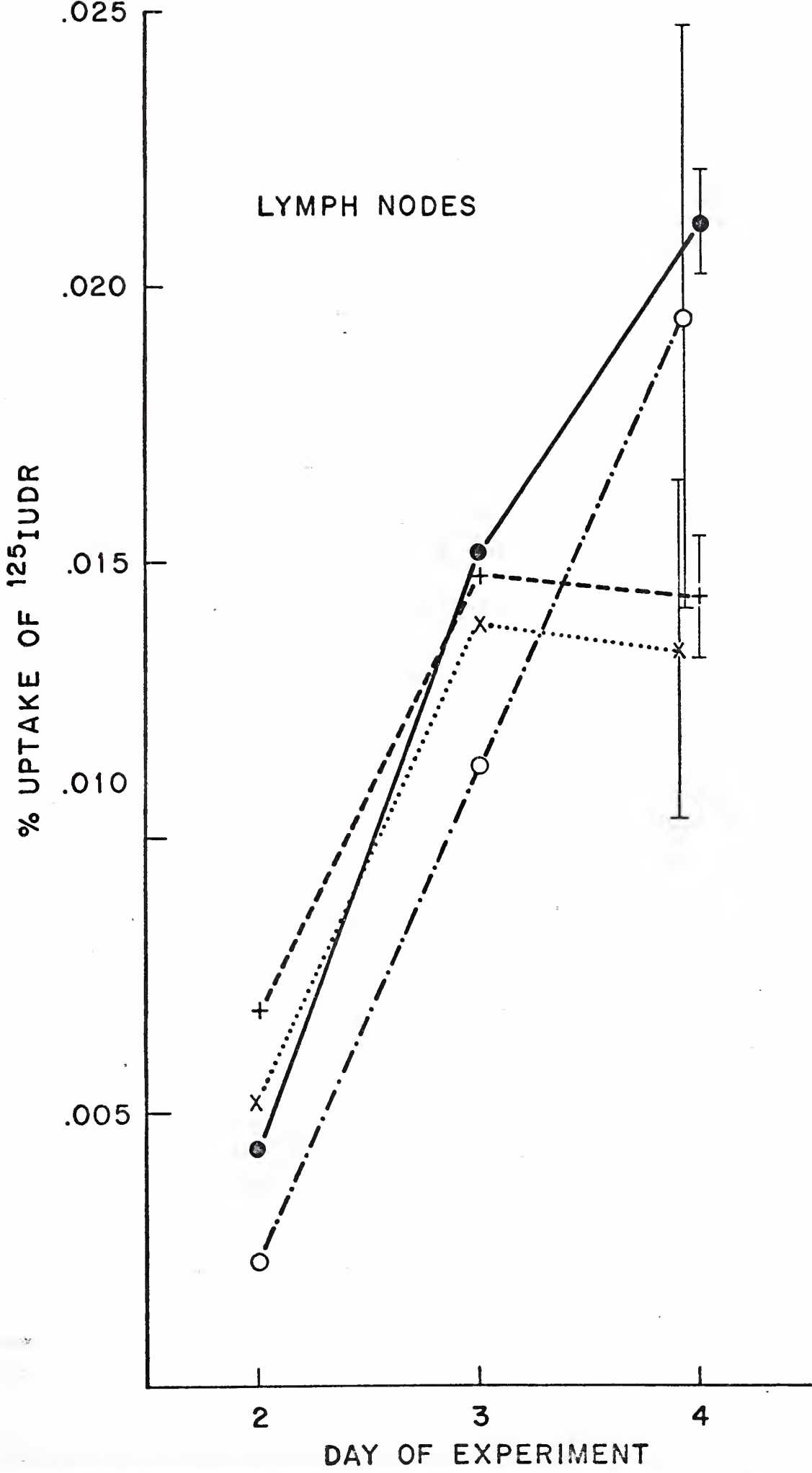
In one experiment (not shown) the addition of irradiated Fl thymocytes to cortisone resistant thymocytes, in the above graft-versus-host system, had no apparent effect on uptake.

C. Response to SRBC in the Presence of a Graft-Versus-Host Reaction

In this experiment, allogeneic (C3H) thymocytes from normal and from cortisone treated donor mice were infused into lethally irradiated C3D2Fl recipients and DNA synthesis was assayed as described above. Some of these recipients, in addition, were also given varying numbers of doses of sheep erythrocytes.

As noted previously, cortisone resistant thymocytes in a graft-versus-host reaction were better responders than whole thymocytes, but not so strikingly as they were in response to SRBC alone (see preceding). When SRBC was added to the graft-versus-host reaction, however, the response of the cortisone resistant thymocytes was seen to be markedly enhanced (Figure 8), while the untreated thymocytes showed little change in splenic uptake of radioisotope.

Lymph node results indicated that, while the cortisone resistant cell uptake in lymph nodes was greatly increased by two doses of antigen, the administration of a third dose decreased uptake to baselike levels (Figure 8). Uptake of isotope by untreated thymocytes did not appear to change with addition of antigen.

FIGURE 8: Ratios of uptake, per cell, of cortisoen resistant:
normal thymocytes, in response to varying doses of SRBC in the
presence of a graft-versus-host reaction (C3H vs. CDF1).

TABLE 6 - Splenic uptake of ^{125}IUDR with multiple doses of antigen in the presence of a GVHR

No. Thymocytes	Cortisone Treated	% Isotope Uptake ± SD	% Uptake Per Cell	SRBC Administration	Day of Exp.
4.5 X 10^7	no	0.0247± 0.0050	5.4X10^{-10}	none	2
		0.0542± 0.0177	12.0		3
		0.0207± 0.0010	4.6		4
	Overall Uptake	0.0996	22.1		2, 3, 4
3.15 X 10^6	yes	0.0125± 0.0045	3.96X10^{-9}	none	2
		0.0239± 0.0026	7.58		3
		0.0130± 0.0010	4.12		4
	Overall Uptake	0.0494	15.7		2, 3, 4
4.5 X 10^7	no	0.0345± 0.0014	7.66X10^{-10}	Days 0, 1	2
		0.0472± 0.0017	10.4		3
		0.0262±	5.8		4
	Overall Uptake	0.1078	24.0		2, 3, 4

TABLE 6 (Continued)

No. Thymocytes	Cortisone Treated	% Isotope Uptake ± SD	% Uptake Per Cell	SRBC Administration	Day of Exp.
3.15×10^6	yes	0.0274± 0.0060	8.69×10^{-9}	Days 0, 1	2
		0.0522± 0.0121	16.5		3
		0.0139± 0.0091	4.41		4
	Overall Uptake	0.0935	29.6		2, 3, 4
4.5×10^7	no	0.0587± 0.0129	13.0×10^{-10}	Days 0, 1, 2	3
		0.0334± 0.0026	7.42		4
	Overall Uptake	0.0921	20.5		3, 4
3.15×10^6	yes	0.0372± 0.0143	11.8×10^{-9}	Days 0, 1, 2	3
		0.0232± 0.0030	7.36		4
	Overall Uptake	0.0604	19.2		3, 4

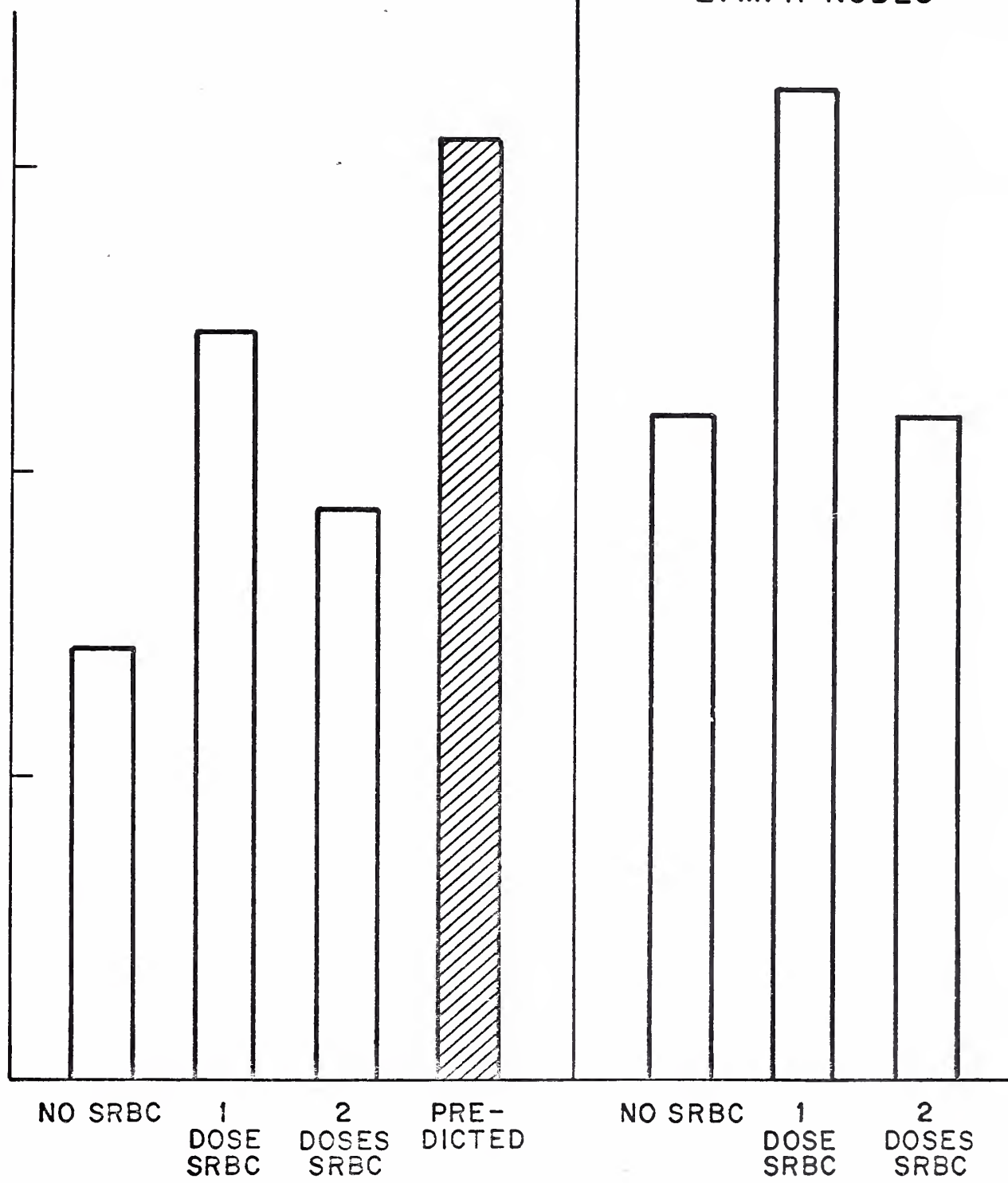TABLE 7 - Femoral lymph node uptake of ^{125}IUDR with multiple doses of antigen in the presence of GVHR

No. Thymocytes	Cortisone Treated	SRBC Administration	Day of Exp.	% Uptake Per Cell	% Isotope Uptake ± SD
4.5×10^7	no	none	2	4.16×10^{-11}	0.00187 ±0.00113
			3	6.64	0.00299 ±0.00293
			4	4.04	0.00182 ±0.00117
	Overall Uptake		2, 3, 4	14.84	0.00678
3.15×10^6	yes	none	2	4.34×10^{-10}	0.00137 ±0.00387
			3	6.44	0.00203 ±0.00084
			4	5.49	0.00366 ±0.00182
	Overall Uptake		2, 3, 4	16.27	0.00513
4.5×10^7	no	Days 0, 1	2	5.09×10^{-11}	0.00229 ±0.00099
			3	8.04	0.00362 ±0.00088
			4	7.26	0.00327 ±0.00020
	Overall Uptake		2, 3, 4	20.4	0.00918

TABLE 7 (Continued)

No. Thymocytes	Cortisone Treated	% Isotope Uptake ± SD	% Uptake Per Cell	SRBC Administration	Day of Exp.
3.15×10^6	yes	0.00337 ±0.00150	10.7×10^{-10}	Days 0, 1	2
		0.00573 ±0.00308	18.2		3
		0.00139 ±0.00053	4.41		4
	Overall Uptake	0.01049	33.3		2, 3, 4
4.5×10^7	no	0.00333 ±0.00072	7.40×10^{-11}	Days 0, 1, 2	2
		0.00282 ±0.00073	6.26		3
	Overall Uptake	0.00615	13.7		3, 4
3.15×10^6	yes	0.00244 ±0.00152	7.74	Days 0, 1, 2	3
		0.00226 ±0.00101	7.17		4
	Overall Uptake	0.00470	14.9		3, 4

IV. DISCUSSION

1. Relative Responsiveness of Untreated versus Cortisone Resistant Thymocytes

Whereas previous investigators (56, 60, 61, 63 - 65) have favored the view that cortisone resistant cells represent the only immunocompetent intrathymic population, evidence is presented herein that their relative contribution to total thymus immuno-competence varies according to experimental conditions.

In particular, while cortisone resistant thymocytes account for most of the thymocyte DNA synthetic response to sheep erythro-cytes (Figure 1), they make up only about half the total thymic response in a graft-versus-host reaction (Figure 5). These ob-servations are in accord with the experiments of Blomgren and Andersson (56) who reported that cortisone resistant thymocytes were only half as reactive in inducing graft-versus-host spleno-megaly as would be expected were they the sole reacting cells in the thymus. Similarly, the results are also in agreement with the work of Andersson and Blomgren (60) who showed data indicating that cortisone resistant thymocytes were 96% as active in restoring sheep hemolysin response in thymectomized, lethally irradiated, bone marrow reconstituted mice as would be expected if they alone

among thymus cells were capable of responding to antigen.

Cortisone resistant thymocytes, then, do not perform as well in graft-versus-host reactions as would be predicted, but they do respond almost as well as would be predicted in response to SRBC. The explanation of these observations may be that cortisone sensitive thymocytes make significant contributions to the DNA synthetic response in the graft-versus-host system. The major histocompatibility antigens are far more antigenic than heterologous erythrocytes, hence it would be reasonable to expect any immunocompetence that these cells might possess to show up best in response to the H-2 antigens. The failure of cortisone resistant cells to account for 100% of the DNA synthesis in response to SRBC may also indicate that cortisone sensitive cells contribute in a small way to this reaction.

2. Different Patterns of Response to Antigenic Stimuli in Untreated and Cortisone Resistant Thymocytes

In this report, numerous instances are shown in which cortisone resistant thymocytes behave differently than thymocytes derived from untreated donors. In particular, they appear to be a far more labile population.

For instance, cortisone resistant thymocytes show greatly augmented responses to repeated challenge with sheep erythrocytes

(Figure 1) while the response of untreated thymocytes under the same conditions is relatively unchanged.

Similarly, in a graft-versus-host reaction, cortisone resistant thymocytes are seen to respond markedly to the presence of added SRBC, while untreated thymocytes do not (Figure 8). When three doses of SRBC are given instead of two, the cortisone resistant thymocyte peak response in lymph nodes is diminished.

The labile response of cortisone resistant thymocytes, contrasted with the response of untreated thymocytes, may be interpreted as indirect evidence for interactions between the cortisone sensitive thymocyte subpopulation and the cortisone resistant subpopulation. The presence of cortisone sensitive thymocytes, in all instances studied, appears to exert a stabilizing, perhaps regulatory effect on the highly responsive cortisone resistant cells. If antigenic stimuli are powerful enough the cortisone sensitive thymocytes may be capable themselves of responding. This phenomenon may be related to the proposed T1 to T2 transition model proposed by Raff and Cantor (80).

3. Localization of Cortisone Resistant Thymocytes

These experiments showed some evidence (Table 5) that cortisone resistant thymocytes preferentially localized in lymph nodes,

as judged by the uptake of isotope in lymph nodes during the
course of a graft-versus-host experiment. This observation is
in accord with the Cr^{51} localizing studies of Lance and Taub
(78) and Lance and Cooper (79), where it was found that cortisone
resistant cells indeed did show a tendency to seek out and re-
main in lymph nodes.

4. Interaction between thymocyte populations: Direct Evidence

In several experiments evidence is presented to suggest
interactions between thymocytes.

First evidence will be considered for positive, "synergistic"
interactions. This form of cooperation is difficult to prove in
the ^{125}IUDR uptake system: whether or not it is valid to expect
the responses of individual cell populations to add arithmetically
when these cells are mixed together and infused is not yet deter-
mined. Indeed, initial experience with this system, reported in
part by Gershon and Hencin (75), suggested that the splenic iso-
tope uptake is not a linear function of cell dose, i.e., that
doubling the number of infused cells results in considerably less
than twice the splenic isotope uptake. Furthermore, at least in
SRBC experiments, "background" response -- that is, uptake in the
absence of antigen -- accounts for a significant but variable

proportion of total uptake, depending mostly upon the absolute value of the latter. Background undoubtedly also is in part due to as yet unknown or uncontrollable variable, such as DNA synthesis in response to infection in the lethally irradiated animal.

Keeping in mind, then, the inherent unresolved mathematical problems in interpreting these data, attention is directed toward the apparent additive effects on total DNA synthesis that result from combining thymocyte populations (Figure 3). These are not suggestive of cooperation, but rather can probably be explained simply on the basis of arithmetical addition of the individual responses. However, it must be emphasized that synergistic interactions are by no means ruled out by these experiments.

Suppressive, or "antergistic" interaction is easier to demonstrate. This is assumed to have occurred when the combining of two cell populations results in a combined response less than that of one of the individual populations. In the SRBC system, this phenomenon was suggested in several experiments and was convincingly demonstrated in one (Figures 6 and 7). In graft-versus-host experiments, it appears that Fl thymocytes can exert suppressive effects (S. Liebhaber, unpublished data). Cortisone resistant Fl thymocytes in these studies were even more suppressive and in vitro irradiated cortisone resistant thymocytes were among

the most suppressive cell populations yet studied. Perhaps significantly, in all instances where suppression was observed, this phenomenon occurred on or after the peak day of response.

The significance of interactions between thymocytes is difficult to interpret at the present time. It is perhaps most useful to think of thymocyte populations as capable of bidirectional interactions. It is not possible at this point to say whether individual cells may interact in a unidirectional or bidirectional manner.

The experiments reported herein shed little light on the actual mechanisms of thymocyte-thymocyte interactions, but whatever the explanation turns out to be, certain generalizations may be drawn from these and other data:

The cortisone resistant thymocyte population seems to represent a more labile cell population with entirely different behavior than whole thymocytes in response to antigens. The presence of suppressed first order kinetics suggests that these cells may be capable of feedback inhibition of themselves. In other experiments, they appear to be capable of suppression of whole thymocytes, and this suppression is potentiated by _in vitro_ irradiation of the cortisone resistant thymocytes.

The cortisone sensitive thymocytes appear to exert some kind of regulatory influence on cortisone resistant thymocytes as judged by the marked differences in response to antigen between combined populations and isolated cortisone resistant thymocytes. In response to a relatively weak antigenic stimulus (SRBC), the cortisone sensitive thymocytes seem to confine themselves to regulation of the cortisone sensitive cells; in the presence of potent histocompatibility antigens, the cortisone sensitive cells seem to be themselves responding as well as playing a regulatory role.

In vitro irradiation of thymocytes appears to enhance their suppressive effect on other thymocytes. It has been shown that cooperation of immunized T-cells with B-cells in the restoration of hemolysins is radiation resistant (81), but no data is yet published to suggest that immunosuppressive actions of T-cells are radiation resistant. Such radiation resistance would point to either a persistent chemical immunosuppressant (?IgY) or the selection by irradiation of an extraordinarily radiation resistant immunosuppressive cell. Alternatively, the possibility exists that the effect of radiation is to reduce the background DNA synthesis of the suppressor cells, hence resulting in a reduced total DNA synthesis.

An overall interpretation of thymocyte interactions at this point is not possible, other than to say that they appear quite complex. It seems clear that at least some thymocyte populations are capable of bidirectional interactions. The cortisone sensitive population appears to be a highly labile intrathymic cell fraction, is capable of vigorous response to antigen and may in turn be controlled by cortisone sensitive thymocytes an by feedback control upon itself. Though positive interactions between cortisone resistant thymocytes and other cells have not been shown in these experiments, these have not been excluded and it would seem teleologically reasonable to seek these out.

5. Possible Physiologic Role of Corticosteroids in Immunity

In conclusion, a possible role for corticosteroids in the regulation of overall immune responsiveness is proposed. It is recognized that stress and its accompanying increased cortisol secretion rate have far-reaching effects on immune reactivity (83, 84), increasing or decreasing the immune response depending on experimental conditions. Indeed, Dougherty et al. (5) have proposed that varying rates of corticosteroid secretion may be an important regulatory influence on the "maturation" rate of lymphatic tissue. Blomgren and Andersson (64) have shown differential responsiveness of thymic cell populations in the process of recovery from corticosteroid administration. It is hereby speculated that the observed effects of corticosteroids on the immune system may be due to their lytic effects on a cortisone-sensitive, regulatory cell population in or outside of the thymus.

Recent experiments (reported in 80) have shown that there is an acute deficit of a cell type (T1) in the spleens of adult thymectomized mice. Perhaps this is the cortisone sensitive thymocyte whose effects have been measured in the experiments presented herein. If this is true and if the regulatory role suggested is real, then this could help explain the increased

occurrence of autoimmune phenomena in aged mammals whose thymuses

have involuted and who consequently have a deficiency of these

cells.

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