part, participate in inducing the secondary rise of plasma NEFA following the injection of nicotinic acid.

Summary. Serial plasma glucose, NEFA, and HGH concentrations were measured in normal male subjects following saline injection (Group 1, 9 cases), nicotinic acid injection (Group 2, 5 cases), and nicotinic acid plus heparin injection (Group 3, 4 cases) for 180 minutes. There was no appreciable change of plasma glucose in all groups. In Group 1 there was no significant change of plasma, NEFA and HGH. In Group 2, plasma NEFA showed an initial decrease followed by the secondary rise at 180 minutes, and plasma HGH showed a marked rise at 120 minutes and/or at 180 minutes. In Group 3, plasma NEFA did not show significant reduction and plasma HGH showed no significant changes. From the results obtained, it was suggested that the lowering of plasma NEFA levels by nicotinic acid administration can stimulate the secretion of HGH, and an assumption was made that plasma NEFA could be at least one of the factors in regulating HGH secretion. It was also suggested that plasma HGH may, at least in part, participate in inducing the secondary rise of plasma NEFA following the injection of nicotinic acid.

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Inhibition of the Lymphopenic Effect of Cortisol by Puromycin Injection in Mice.* (32550)

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The lymphopenic action of adrenal cortical steroids was established a number of years ago, and is well documented(1,2). At 3 to 6 hours following a single injection of an effective steroid, blood lymphocytes values are depressed to a minimum. Stimulation of the pituitary-adrenal axis by any one of a wide

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variety of agents also causes a lymphopenia in intact, but not adrenalectomized animals (1,2). It was postulated that the lymphopenic response to lymphocytokaryhorrectic steroids is a result of the failure of delivery of normal numbers of lymphocytes to the blood from the lymphoid tissues consequent to the dissolution of lymphocytes in lymphoid organs(1-3). However, the biochemical basis of the alterations in lymphoid tissue structure and function produced by pituitary-adrenal cortical secretory activity is unknown. The present study was initiated to determine whether the administration of puromycin, an agent known to depress protein synthesis *in vivo*(4), would affect the lymphopenic action of cortisol.

Puromycin has been used to study a number of responses to glucocorticoids in order to ascertain whether these are correlated with a requirement for protein synthesis. Among these responses are the enhancement of ribonuclease in a lymphoid tissue(5-7), and the depression of purine and ribonucleic acid synthesis in thymus(8). In addition, the manifold anabolic effects of glucocorticoids in liver have been examined for sensitivity to puromycin. These include increases in enzymatic activity(9-12), synthesis of purines, ribonucleic acid(8,12) and carbohydrate(12,13), and enhancement of glycogen storage(12,14). However, it has been noted(15-17) that the use of puromycin as a means of studying hormone action may be complicated by the fact that there may be effects of puromycin, such as its glycogenolytic activity(18, 19), that are not a result of inhibition of protein synthesis. Attempts have been made to circumvent this problem by using, in lieu of puromycin, 6-dimethylaminopurine (6-DAP), a puromycin analogue that does not depress protein synthesis but is glycogenolytic(13,19-22). This approach has been used in the present study with the finding that treatment with puromycin, but not with 6-DAP, will prevent the lymphopenic action of cortisol. The data suggest that protein synthesis is an essential step for the expression of this response to glucocorticoids.

Materials and methods. CBA male mice, raised in our laboratory and given laboratory chow and water *ad libitum*, were used at 6-7 weeks of age, either intact or adrenalectomized. The latter group was bilaterally adrenalectomized under ether anesthesia, given 0.9% NaCl to drink, and used 18-24 hours postoperatively. Saline or cortisol in saline suspension was given intraperitoneally as a single injection at zero time. Because the inhibitory effect of puromycin on protein synthesis is somewhat transient(18), it is of importance that a significant depression of lymphocytes can be measured at early times after cortisol treatment. Puromycin (300 mg per kg dissolved in 1.38% $NaHCO_3$) was injected intraperitoneally in 3 divided doses, one-half simultaneously with the steroid and the remainder in equally divided doses at 1 and 2 hours. Controls were either uninjected or received a volume of vehicle equal to that given to the experimental animals. Total white blood cell values and differential counts on at least 200 cells stained by Wright's stain were determined at 3 hours following cortisol injection. Monocytes and lymphocytes were treated as a single population, and are referred to as lymphocytes.

Results and discussion. Table I shows that treatment of intact or adrenalectomized mice with $NaHCO_3$ and saline caused a depression of the absolute number of lymphocytes. Although this response might be anticipated in the intact animals as a result of stimulation of the pituitary-adrenal secretory mechanism, the lymphopenia in the adrenalectomized animals was surprising. However, despite the lymphopenia produced by administration of vehicle alone, cortisol injection resulted in a further significant absolute lymphopenia in both intact ($p<0.001$) and adrenalectomized mice ($p<0.01$).

Lymphocyte values of adrenalectomized animals treated with puromycin were elevated above those of mice that either received vehicle alone or were untreated. This response is in agreement with the documented lymphocytosis of stressed-adrenalectomized animals(23). Because of the toxicity of puromycin(24), it might be expected that lymphopenia would ensue in the intact animals as a consequence of adrenal stimulation and release of corticosteroids(1,2). It can be seen, however, that puromycin treatment of the

TABLE I. Inhibition of the Lymphopenic Effect of Cortisol by Puromycin Treatment of Mice.

Treatment*	Animals (No.)	Leucocytes (cells/mm³) Total	Lymphocytes	Polymorphonuclear
		Intact		
None	12	9,958 ± 520	6,704 ± 477	3,254 ± 300
$NaHCO_3$, saline	12	9,271 ± 713	3,182 ± 203	6,088 ± 602
$NaHCO_3$, cortisol	12	5,346 ± 264	1,953 ± 169†	3,389 ± 71†
Puromycin, saline	12	12,215 ±1,005	6,364 ± 759†	5,850 ± 657
Puromycin, cortisol	11	12,141 ± 731	5,678 ± 546‡	6,463 ± 761
		Adrenalectomized		
None	12	9,391 ± 699	6,193 ± 391	3,198 ± 442
$NaHCO_3$, saline	12	7,400 ± 887	2,862 ± 322	4,538 ± 577
$NaHCO_3$, cortisol	12	5,746 ± 403	1,554 ± 294§	4,192 ± 372
Puromycin	11	19,206 ±1,883	9,925 ±1,085	9,281 ± 987†
Puromycin, cortisol	11	19,191 ±1,657	10,823 ± 979	8,363 ±1,000

* One-half of the total puromycin (300 mg/kg) was given at zero time, and one-fourth of the total, at 1 and 2 hr. Cortisol was administered at zero time at a dosage of 0.5 mg per mouse for intact mice, and 1.0 mg per mouse for adrenalectomized animals. All solutions were given intraperitoneally and the blood sampled at 3 hr.

† Compared to value for $NaHCO_3$, saline treatment, $p < 0.001$. All values in this table are means ± standard error of means.

‡ Compared to value for puromycin, saline treatment, $p > 0.4$.

§ Compared to value for $NaHCO_3$, saline treatment, $p < 0.01$.

intact mice did not result in a depression of the numbers of lymphocytes, since lymphocyte counts were the same as those for untreated animals. In addition, elevating the serum glucocorticoid level by cortisol injection failed to depress the number of blood lymphocytes in either intact or adrenalectomized mice treated with puromycin. Thus, puromycin administration did not permit expression of the lymphopenic action of the steroid, whether injected or released endogenously. The absence of a stressed-induced lymphopenia in the intact mice following puromycin injection may also be a result of a puromycin inhibition of the stimulation of adrenal steroid secretion by adrenocorticotropic hormone(25).

Inasmuch as peripheral lymphocyte values might be altered by hemoconcentration, hemoglobin was determined on cardiac blood in a typical 3-hour experiment. The values obtained in milligrams per 100 ml of blood for four groups of adrenalectomized mice were as follows: control, 14.1; cortisol, 12.5; puromycin, 16.5; and puromycin plus cortisol, 14.5. Thus apparent minor alterations in blood volume could not be responsible for the puromycin-induced inhibition of cortisol action. It is important to note that the effect of puromycin is not that of inducing a lymphocytosis that can be overcome by corticosteroid injection. Such an action conceivably could result in no change in the absolute number of lymphocytes as compared to control values. In this context, lymphocytotic responses induced by agents such as heparin and pertussis vaccine are sensitive to glucocorticoid treatment(26,27).

Experiments designed to determine whether puromycin treatment of mice would inhibit lymphoid tissue involution, as reflected in alteration of weight of the thymus, were equivocal. Adrenalectomized mice, which must be used in a study of this type, do not tolerate the toxicity of puromycin for extended periods of time.‡ In one experiment, administration of 100 mg of puromycin per kilogram to adrenalectomized mice every 4 hours for a 16-hour period caused only one death in a group of 6 animals. This treatment did not inhibit the cortisol-induced involution of thymus, spleen and lymph nodes. However, because of the rapid clearance of

‡ Although the dosage of 300 mg/kg given in a single injection caused no deaths in the intact mice (LD_{50} for intraperitoneal puromycin in mice is 580 mg/kg; J. F. Sherman, D. J. Taylor, H. W. Bond, Antibiot. Ann. 757, (1954-1955), a high mortality was observed in the adrenalectomized animals following the 3 hour period of observation. This effect of puromycin could be overcome by injection of cortisol.

puromycin from the blood, it is uncertain to what extent this dosage of puromycin would inhibit protein synthesis for that protracted period of time(18). Puromycin injection itself caused no significant depression of lymph node or thymus weight, whereas the weight of the spleen was depressed by the end of the 16-hour period. The effect of puromycin on splenic weight has been reported(28). Halkerston *et al*(29) have reported that actinomycin D injection did not inhibit the ability of cortisol to cause thymic involution. Blood lymphocyte values were not measured in that study.

It has been shown that puromycin injection causes rapid liver glycogen depletion independent of its effect on protein synthesis. Since it was possible that puromycin blocked the lymphopenic activity of cortisol in a manner not related to its inhibitory action on protein synthesis, 6-DAP, the puromycin analogue that has glycogenolytic activity but does not affect protein synthesis(19), was tested as a possible inhibitor of the cortisol-induced lymphopenia. Table II shows that cortisol injection in adrenalectomized mice caused a significant reduction in blood lymphocytes at 3 hours, but that administration of 6-DAP failed to block the response to cortisol. This dose of 6-DAP was shown previously to be sufficient to deplete hepatic glycogen of mice(19). These results suggest that the inhibitory effect of puromycin administration upon the lymphopenic action of cortisol is based upon the ability of puromycin to inhibit protein synthesis.

An important question raised in these studies is the site at which puromycin may act in blocking the lymphopenic activity of cortisol. Puromycin has been shown to cause inhibition of protein synthesis in lymphoid tissue(4,24), and to prevent the influx of lymphocytes into sites of inflammation(30). On the other hand, because puromycin could be considered to be an hepatotoxic agent(18), and since it has been shown that hepatectomy inhibits the lymphopenic effect of cortisol (31), it is possible that some anabolic effect in the liver following glucocorticoid treatment is requisite for the lymphopenic response.

TABLE II. Effect of 6-Dimethylaminopurine (6-DAP) on Lymphopenic Action of Cortisol in Adrenalectomized Mice.

Treatment*	Animals (No.)	Leucocytes (cells/mm³)		
		Total	Lymphocytes	Polymorphonuclear
Saline	6	7,442 ± 462	3,462 ± 248	3,979 ± 412
Saline, cortisol	6	8,467 ± 971	1,954 ± 104†	6,513 ± 902
6-DAP, saline	7	12,400 ±1,812	5,213 ± 935	7,186 ± 971
6-DAP, cortisol	6	10,058 ±1,307	2,668 ± 389‡	7,390 ±1,338

* The 6-DAP (total dosage 130 mg/kg) was given in the same manner as puromycin (Table I). Cortisol (1.0 mg per mouse) was administered with the initial injection of 6-DAP, and blood samples taken at 3 hr.

† Compared to value for saline treatment, $p < 0.001$. All values in this table are means ± standard error of means.

‡ Compared to value for 6-DAP, saline treatment, $p < 0.05$.

The synthesis of glutamate and its release from the liver as a consequence of enhanced transaminase activity have been suggested as a mechanism for the role of the liver in glucocorticoid action on lymphoid tissue(32). However, recent evidence suggests that a heat-labile factor of liver origin may be of significance for mediation of these effects of cortisol(33).

Summary. In acute experiments, administration of puromycin to adrenalectomized or intact mice inhibited the lymphopenic action of cortisol, whereas injection of 6-dimethylaminopurine, a puromycin analogue, did not prevent this response to cortisol. It is suggested that protein synthesis is an essential preliminary or accompanying process for the manifestation of the lymphopenic activity of glucocorticoids.

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Transport and Storage of ^{14}C-Riboflavin in the Retina and Liver of Rats.* (32551)

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Riboflavin has been reported to exist in significant amounts as the free vitamin in parts of the eyes of various animals(1-3) in contrast to such other tissues as liver where the coenzyme forms; riboflavin-5′-phosphate (FMN) and especially flavin adenine dinucleotide (FAD), predominate(4). Riboflavin has been found to occur in the pigmented epithelium of fish eyes(1), as crystals in the tapetum of a particular species of lemur(2) and in choroid and iris of frogs(3). FMN has been found in frog retina(3) and FAD has been detected in ox retina(5). An unidentified flavin has been isolated from choroid of cats(6). Uptake of dietary riboflavin into corneas was demonstrated with riboflavin-deficient rats(7).

The present study was undertaken to follow the uptake and storage of injected riboflavin by retina as compared with liver of deficient and control rats. A further purpose was to compare the predominant form of flavin in these tissues.

Materials and methods. Riboflavin was

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