TABLE I. *Hymenolepis nana* Infection in X-Irradiated Mice Implanted with Bone Marrow. Each entry indicates the number of cysticercoids (larvae) in a single mouse 93 to 96 hours after post-irradiation challenge with parasite eggs.*

Nonirradiated (controls)		Immunized & irradiated (950 r)		
		Syngeneic marrow		Rat marrow
Nonimmunized	Immunized	40×10^6 cells	1×10^6 cells	204×10^6 cells
50	0	5	82	157
143	0	0	92	126
311	0	0	0	126
350	0	0	26	83
236	0	0	141	295
164	1	0	6	239
213	0	0	226	301
185	0	12	27	203
176	0	0	12	313
342	0	0	134	301
		0	22	382
		0	82	338
		0	0	319
		0	31	
		0	92	
			20	
			2	

* Mice immunized with 14×10^3 parasite eggs 67 days before irradiation. Preirradiation challenge with 7.6×10^3 eggs 21 days before irradiation. Postirradiation challenge with 4.8×10^3 eggs 22 days after irradiation. Nonimmunized control mice were given only the "postirradiation" egg dose; immunized controls received the immunizing dose and the two challenge doses of parasite eggs.

the transplanted syngeneic marrow—the more donor marrow the more immunologically competent cells and, therefore, the greater opportunity for transfer.

Summary. X-irradiated mice preimmunized to *Hymenolepis nana* were injected with either 1 million or 40 million syngeneic bone-marrow cells from nonimmune mice or with 204 million rat marrow cells. Twenty-two days after irradiation the mice were given *H. nana* eggs. Infection rates were lowest in the animals injected with the larger number of syngeneic marrow cells; the mice implanted with rat marrow had the highest infection rates. We conclude that transplanted syngeneic marrow from nonimmune donors makes less severe (or prevents) a radiation-induced depression of acquired immunity.

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Effect of Corticosteroids on Amino Acid Incorporation into Protein by Rat Liver Ribonucleoprotein Particles. (31855)

FRANK DEVENUTO AND ROBERT J. G. LANGE
Biochemistry Division, U. S. Army Medical Research Laboratory, Fort Knox, Ky.

The effects of glucocorticosteroids and other steroid hormones on protein synthesis, as evaluated by amino acid incorporation, have been studied by several investigators and a variety of tissues have been used(1,2).

In a previous paper(3) on the incorporation of leucine-C^{14} into protein, with cell-free preparations from rat liver, it was shown that the ribonucleoprotein (RNP) particles, prepared from liver of adrenalectomized rats up to 15 days after adrenalectomy, have a greater incorporating activity than the corresponding particles from liver of normal rats. The present study was undertaken to investigate the amino acid incorporating activity of these liver particles after *in vivo* administration of exogenous corticosterone to the normal and adrenalectomized animals and after *in vitro* addition of corticosterone to the incubation system. An attempt was also

made to separate by starch-electrophoresis the labeled protein(s) obtained after incubation.

Experimental. Materials. Uniformly labeled L-leucine-C^{14} with a specific activity of 240 mc/mmole was obtained from New England Nuclear Corp.; crystalline pyruvate kinase from California Corp. for Biochemical Research; ATP, GTP from Pabst Laboratories; phosphoenol-pyruvate and desoxycholic acid (sodium salt) from Sigma Chemical Co. and Mann Research Laboratories, respectively; puromycin (tetracycline HCl) from Lederle Laboratories. Male Sprague-Dawley hooded rats of 200-250 g weight, bred in our laboratory, were used for these experiments.

Preparation of particles and incubation. The animals were decapitated and exsanguinated, the abdominal cavity opened and the livers were quickly removed and homogenized in a medium containing 0.35 M sucrose, 0.025 M KCl, 0.01 M $MgCl_2$, 0.035 M Tris-HCl buffer, pH 7.5, 0.005 M β-mercaptoethanol. The preparation of RNP-particles and of pH 5-enzymes was carried out essentially according to the procedure of Liao and Williams-Ashman(4) and of Kirsch *et al*(5) and as outlined previously(3). The RNP-particles and the pH 5-enzymes, if not used immediately, were stored in a deep-freeze at —120°F. The incubation system and the various components used for the study of L-leucine-C^{14} incorporation with pH 5-enzymes and RNP-particles are outlined in Table I. The procedures used for the precipitation of protein with trichloroacetic acid (TCA) at termination of the incubation, the purification and washing of the precipitate protein and the determination of radioactivity have also been reported(3).

Starch-electrophoresis. Separation of the labeled protein(s), obtained after incubation and precipitation, was carried out by starch-block electrophoresis. The apparatus used is similar to that described by Kunkel and Slater(6). The solution containing labeled protein(s) was concentrated under nitrogen and mixed with dry potato starch so that the thin slurry which formed could be poured into a rectangular slit cut transversely 5 cm from one end of a starch block 4 × 25 cm and 0.5 cm thick. The starch block was equilibrated with phosphate buffer, pH 7.7, ionic strength 0.1, for 2 hours at 5°C after which an electromotive force of 115 v and 30 ma was applied. After 72 hours the starch block was allowed to dry partially and then partitioned in 1 cm segments. Each segment was eluted with phosphate buffer and the eluates tested for protein content and for radioactivity.

Other procedures. Adrenalectomy was performed under ether anesthesia by the lumbar approach. The adrenalectomized animals were maintained under the same conditions as the control rats except that saline was allowed *ad libitum*. Completeness of the adrenalectomy was verified by visual inspection at the time the animals were sacrificed, 8 days after the operation. Groups of adrenalectomized rats were injected with corticosterone in saline once daily for a total of 3 consecutive days beginning on the fourth day after adrenalectomy; 1 ml saline containing 2 mg of corticosterone was injected subcutaneously. Twenty-four hours after the last injection (8 days after adrenalectomy) the animals were sacrificed; RNP-particles and pH 5-enzymes were then prepared. Another group of operated animals and a group of normal rats were injected with saline and corticosterone, respectively, using the same procedure used for the corticosterone-injected adrenalectomized animals.

Protein determinations on the starch-electrophoresis fractions were done using the method described by Lowry *et al*(7). Proteins isolated after incubation and dissolved in formic acid were quantitatively determined by their optical density at 280 mμ with a Cary recording spectrophotometer. The biuret reaction was used in every other instance; crystalline bovine plasma albumin (Armour Pharmaceutical Co.) was the standard for each procedure.

Results and discussion. The effects of a variety of treatments on the ability of the RNP-particles to catalyze the *in vitro* incorporation of the C^{14} label of amino acids into the protein system are illustrated in Table I. Flasks No. 1 and 2, containing RNP-particles from livers of normal rats, are

TABLE I. Incorporation of Leucine-C^{14} by pH 5 Enzymes-RNP System Using RNP-Particles from Livers of Rats Subjected to Various Treatments.

The complete incubation system contained: 100 μg pyruvic kinase, 10 μmoles phosphoenolpyruvate, 1 μmole ATP, 5 μmoles $MgCl_2$, 1 μmole GTP, 2.25 mμmoles L-leucine-C^{14} (2.5 μc), pH 5-enzyme suspension (2 mg protein, preparation No. D-35) from normal rats, RNP-particles suspension (4 mg protein) as stated in the table, in a final volume of 2.0 ml. The incubation proceeded for 60 min at 37°C.

Flask No.	RNP prep. No.	Treatment	Total mg protein after incubation	Total cpm in protein	Uncorrected cpm/mg protein	Corrected cpm/mg protein
1	D-35	Normal	5.20	31,892	6,133	6,110
2	D-35	Normal (duplicate)	4.83	31,158	6,451	6,428
3	D-35	Normal (TCA added*)	4.50	103	23	—
4	D-21	Adx†	6.15	80,879	13,151	13,128
5	D-25	Adx + B‡	7.70	27,966	3,532	3,509
6	D-26	Adx-sham injected	7.80	97,664	12,521	12,498
7	D-28	Normal + B	7.30	25,849	3,541	3,518
8	D-40	Normal	6.45	35,294	5,472	5,449

* At start of incubation 5 ml of 5% TCA were added.

† Adx, adrenalectomy.

‡ B, corticosterone injected to the animals' prior preparation of RNP-particles (see experimental).

duplicates and the cpm/mg protein are in good agreement. Flask No. 8 contained RNP-particles from livers of a different group of normal rats. Their activity is comparable to that of the particles in flasks No. 1 and 2. Flask No. 3, in which at the beginning of the incubation 5 ml of 5% TCA were added, is a zero-time control; the incorporation obtained with this flask was subtracted from the other results and corrected cpm/mg protein are reported in the last column. RNP-particles from livers of adrenalectomized rats (flask No. 4) incorporate more than twice as much radioactivity into protein as those from normal rats. This effect is abolished by treating the adrenalectomized rats with corticosterone (flask No. 5) but not by sham-injection with saline (flask No. 6). Treatment of normal rats with corticosterone also appears to depress the incorporation by RNP-particles from the livers of these animals (flask No. 7).

In incubation studies in which a pH 5-enzyme suspension from livers of adrenalectomized animals was used with RNP-particles prepared from livers of normal or adrenalectomized rats the results obtained were similar to those reported in Table I. RNP-particles are necessary to catalyze amino acid incorporation into protein; incubation studies in which no RNP-particles were added to the system the amount of radioactivity incorporated was very low (about 90 cpm/mg protein).

The results in Fig. 1 show the effect of incubation time on C^{14} incorporation into the pH 5 enzyme-RNP particles system using RNP-particles prepared from livers of normal or adrenalectomized animals. After 5 minutes there is a substantial amount of radioactivity incorporated into the protein and the values increase with the length of the incubation period. Throughout these time periods studied the RNP-particles from livers of adrenalectomized animals were more active in radioactivity incorporation than the corresponding particles from livers of normal rats. The ratio of $\frac{\text{cpm per mg protein Adx}}{\text{cpm per mg protein Normal}}$ varied from a low of 2.13 to a high of 3.35 with an average value of 2.5.

It has been shown by several investigators (8,9) that puromycin inhibits protein synthesis. The addition of puromycin to an *in vitro* system containing RNP-particles from the rat prostate gland(4) inhibits the incorporation of valine-C^{14} into the protein system by a factor of 10. In our studies, Table II, the addition of 0.2 μmoles of puromycin to the incubation system decreases the radioactivity incorporation by a factor of

10 when RNP-particles from livers of normal rats are used and by a factor of about 7 with RNP-particles from livers of adrenalectomized rats. In Table II it is also shown that direct addition of 5 μg of corticosterone to the incubation flask does not influence the radioactivity incorporated using RNP-particles prepared from livers of normal or adre-

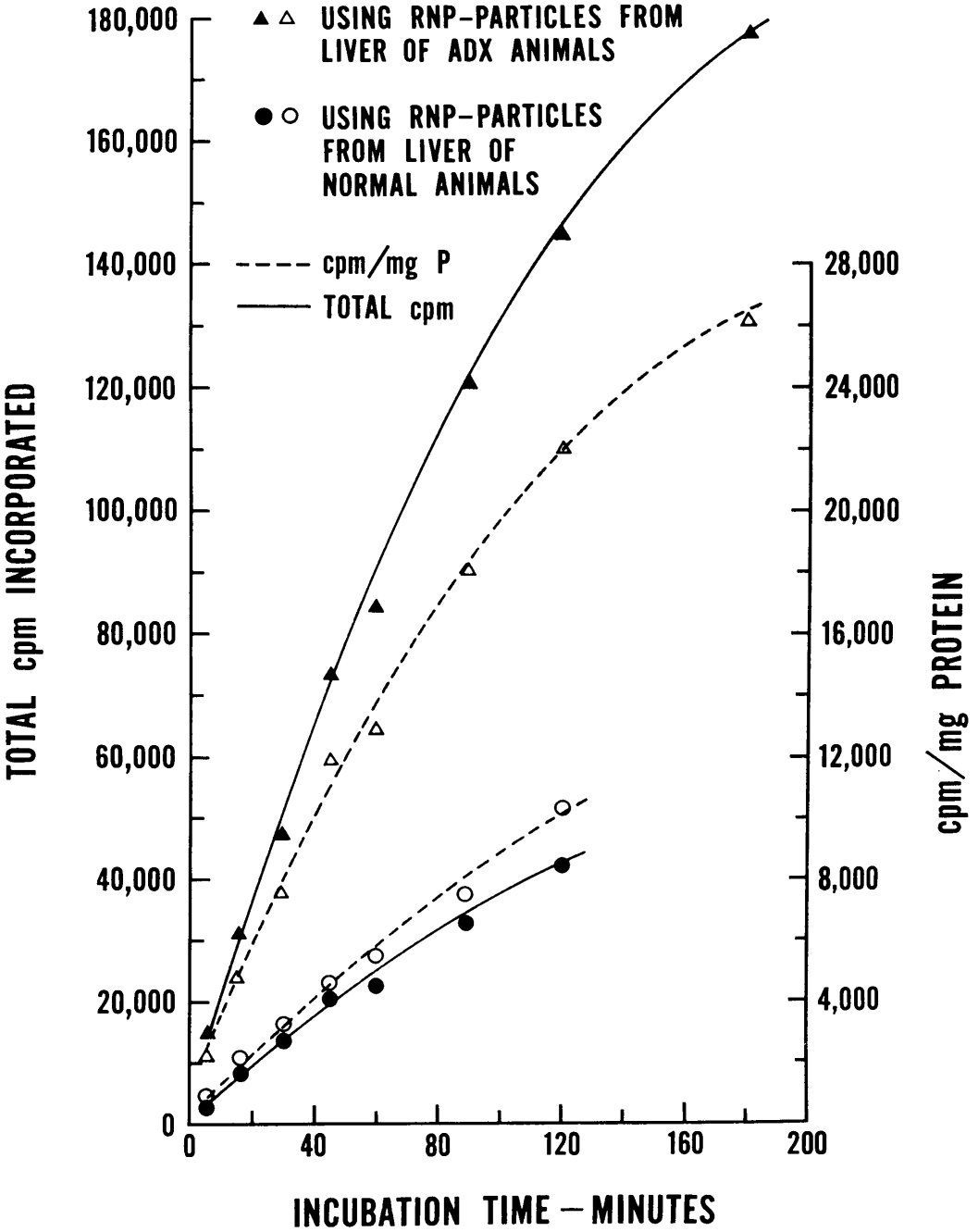

FIG. 1. Effect of time of incubation on C^{14} incorporation. The incubation system is the same as that outlined in Table I, using the pH 5-enzyme suspension from livers of normal rats and RNP-particles from livers of normal or adrenalectomized animals, as indicated.

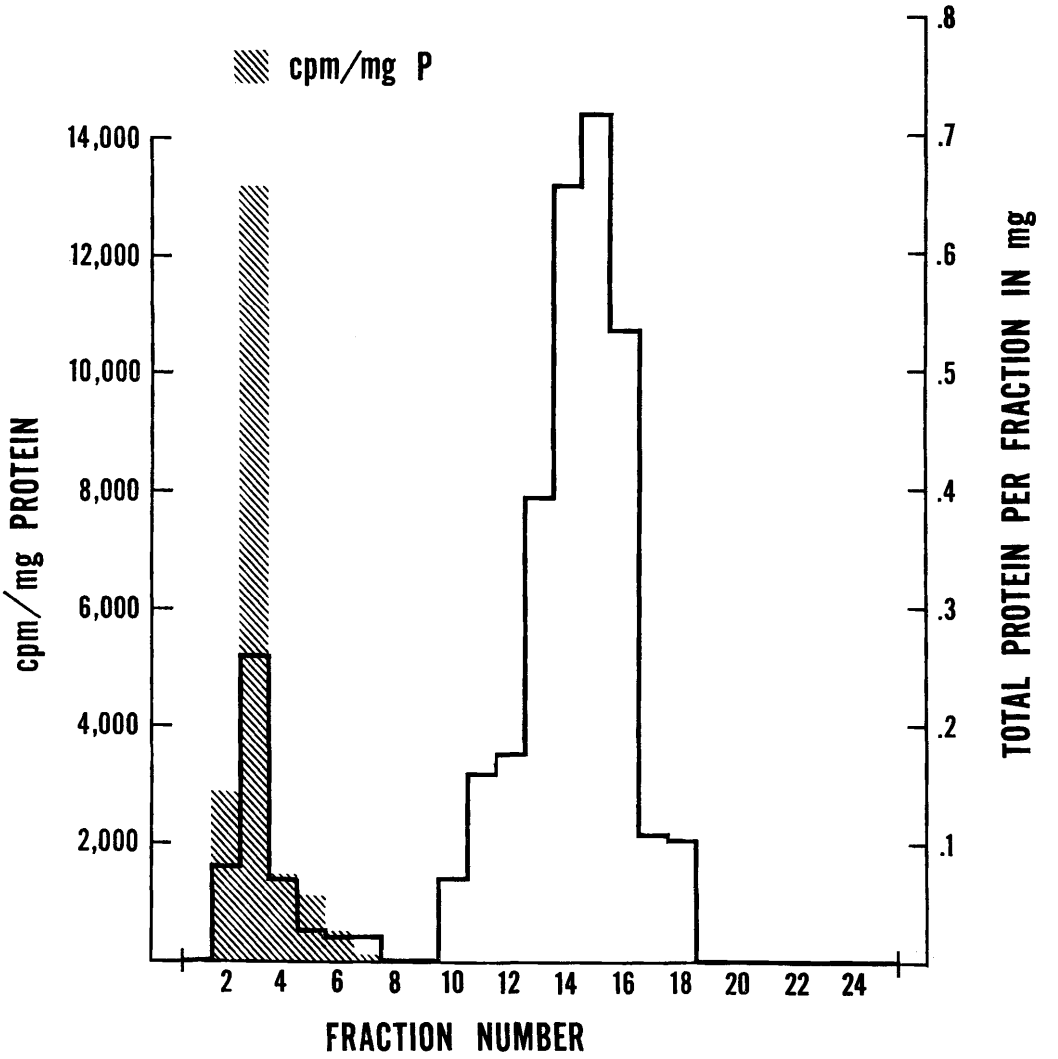

FIG. 2. Starch electrophoresis of labeled protein after incubation. Incubation system as outlined in Table I was used. RNP-particles from livers of adrenalectomized rats and pH 5-enzyme suspension from livers of normal animals were used.

nalectomized rats.

Fig. 2 shows a preliminary attempt to fractionate by starch electrophoresis the labeled protein obtained after incubation. Two protein peaks were obtained; the first peak contained 14% and the second peak 86% of the total protein. The fractions collected were counted to determine the radioactivity content which, as shown in Fig. 2, was completely associated with the first protein peak. Whether during the incubation period the labeled protein is synthesized *de novo* totally or partially cannot now be stated; further studies are under way.

The results show that the ability of RNP-particles to catalyze the incorporation of leucine-C^{14} into protein increases with removal of endogenous corticosteroids from the rat; this increase can be eliminated by administration of corticosterone to the adrenalectomized animals, but not by direct addition of this hormone to the incubation system.

Influences of steroid hormones on amino acid incorporation into protein have been demonstrated by several investigators. Our

TABLE II. Influence of Addition of Puromycin or Corticosterone to the Incubation Flask on Incorporation of L-Leucine-C^{14} by pH Enzymes-RNP System.

Incubation system as in Table I, soon after addition of RNP, 0.2 μmoles of puromycin were added to flasks No. 3 and 6 and 5 μg of corticosterone were added to flasks No. 4 and 7; pH 5-enzyme suspension from livers of normal animals was used in all flasks.

Flask No.	RNP prep. No.	Addition	Total mg protein after incubation	Total cpm in protein	Uncorrected cpm/mg protein	Corrected cpm/mg protein
1	D-40 Normal	None	5.19	38,088	7,338	7,221
2	"	TCA	5.19	608	117	—
3	"	Puromycin	5.23	4,606	880	763
4	"	Corticosterone	5.22	38,526	7,380	7,263
5	D-21 Adx	None	5.65	78,989	13,980	13,863
6	"	Puromycin	5.69	12,572	2,209	2,092
7	"	Corticosterone	5.78	78,969	13,662	13,545

findings are in agreeement with those reported by Korner(10) on the role of the adrenal gland in the control of amino acid incorporation into protein and with the results of Reid and Stevens(11) who reported a small rise in the extent of incorporation of injected amino acid immediately after adrenalectomy of the rat.

Other investigators, however, have reported that glucocorticoids have a stimulating effect on protein synthesis in rat liver. Pena, Dvorkin and White(12) have shown that *in vivo* administration of cortisol to rats stimulates the *in vitro* amino acid incorporating activity of rat liver; these results are in contrast with our findings and the different conditions used might offer a possible explanation. Pena and co-workers investigated early effects of a single dose of cortisol whereas our studies show long range effects of repeated doses of corticosterone.

Summary. The influence of corticosteroids on the incorporation of L-leucine-C^{14} into protein by ribonucleoprotein (RNP) particles from rat liver was studied. When RNP-particles from livers of adrenalectomized animals are used, the C^{14} amino acid incorporated is more than twice that with RNP-particles from normal rats. The increased incorporation is eliminated by administration of corticosterone to the adrenalectomized animals but not by direct addition of this hormone to the incubation system. In the incubation system used, RNP-particles from livers of normal and adrenalectomized rats incorporated C^{14}-labeled amino acid at a rate proportional to the time of incubation. Preliminary electrophoretic separation of the labeled protein resulted in two protein peaks, the first representing 14% and the other 86% of the total protein, with the radioactivity associated entirely with the first protein peak.

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