mogenization on the enzyme induced by either method of induction was comparable to that observed in homogenates of normal livers, except that the degree of inactivation of tryptophan-induced apoenzyme was not related progressively to length of homogenization time. Fig. 4 shows both native holoenzyme and total enzyme activities in unfractionated rat liver homogenates, prepared in the Teflon-glass tissue grinder, during the course of hormonal induction. No basic differences in response during cortisone induction were apparent; however, it is clearly evident that considerably more accurate estimates of actual amounts of tryptophan pyrrolase were obtained when hematin was added to the assay mixture which contained liver homogenates prepared in the Teflon-glass tissue grinder.

With the technique of homogenization described here, it has also been possible to detect tryptophan pyrrolase activity in neonatal rat liver homogenates by the third to fourth day in the absence of agents which chelate copper(6). In addition, free apoenzyme could be detected in unfractionated liver homogenates by the seventh day after birth, which is several days sooner than has been reported previously(7).

Summary. A reservoir of free apotryptophan pyrrolase has been detected in unfractionated rat liver homogenates. The detection of this enzyme fraction is dependent upon its preservation by a gentle technique of homogenizing the liver sample. The activity of free apotryptophan pyrrolase was inactivated when livers were homogenized in a unit with rotor-knife blades.

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Passive Transfer of Allergic Encephalomyelitis: Acceleration by Adrenalectomy.* (30764)

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Adrenalectomy enhances active induction of certain immunologic phenomena including experimental allergic encephalomyelitis (EAE)(1,2). Little is known about the effect of adrenalectomy on passive transfer of immunologic reactions mediated by lymphoid cells, except for a report of enhancement of graft-*vs*-host reaction(3). In the following report, acceleration of passive transfer of EAE by adrenalectomy of recipients will be demonstrated.

Methods. Lewis rats (Microbiological Associates, Inc.) with the hyperacute form of EAE(2) were donors of lymphoid cells(4). For lymph node cell transfers, donors received intraperitoneally 200 mg (wet weight) guinea pig spinal cord and 0.05 ml pertussis vaccine concentrate (10 billion organisms), homogenized and diluted with saline to 3 ml. At the first clinical signs of EAE, donors were sacrificed by exsanguination under ether anesthesia. Mediastinal lymph nodes were removed under sterile conditions, cleaned of fat, minced, and pressed and washed through a Cytosieve with saline. For spleen cell transfers, donors received an intraperitoneal in-

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jection as above, plus identical doses of cord and pertussis vaccine (final volume 0.25 ml) in the sole of each hind foot. Saline suspensions of spleen cells were prepared in the same manner as lymph node cells, except that the former were centrifuged at 2,000 *g* for 10 minutes at 0-4°C and resuspended in saline. All cell suspensions were at least 95% viable according to eosin-Y exclusion test. Suspensions were injected as soon as possible through a 25 gauge needle into the dorsal penile vein of anesthetized intact or adrenalectomized recipient Lewis rats. After sacrifice, entire spinal cord and hindbrain were embedded in paraffin, sectioned, and stained by phosphotungstic acid-hematoxylin for hyperacute EAE (donors) or by hematoxylin-eosin for ordinary EAE (recipients).

Bilateral adrenalectomy of recipients was done under IP pentobarbital plus ether anesthesia through a dorsal midline incision 2 days before passive transfer. This time was determined by a preliminary experiment on active induction of EAE: rats were adrenalectomized at various times before or after injection of 200 mg guinea pig cord—0.05 ml pertussis vaccine concentrate in right footpad; adrenalectomy 2 days before injection gave maximum acceleration of onset of EAE (Table I). (Presumably, adrenalectomy at later times was less effective because of residual corticosteroids circulating during active sensitization.) Adrenalectomized rats were given 1% NaCl as sole fluid. Controls were intact rats from the same shipment, maintained on tap water. Purina Laboratory Chow was fed *ad libitum*.

Results. Pooled cell suspensions from mediastinal lymph nodes of 4 donor rats with EAE were divided in half and injected into 1 adrenalectomized recipient and 1 intact recipient (donor/recipient = 2/1). Seven pairs of recipients were prepared. Clinical signs of EAE (weakness or paralysis) appeared in all adrenalectomized recipients after 2 to 4 days, average 2.7, and in all intact recipients after 4 or 5 days, average 4.3. Each rat was sacrificed on the day after onset of its clinical disease. Adrenalectomized rats had more severe clinical signs than intact rats, but equally severe histologic lesions of EAE were present in all animals of both groups.

TABLE I. Active Induction of EAE: Acceleration by Adrenalectomy.

Day of adrenex	No. of rats	Onset of EAE (avg)
D—9	12	5.3 days
D—2	15	5.1
D—1	10	5.9
D 0*	10	6.2
D+4	10	6.8
None	10	7.7

* Day of injection of cord-pertussis mixture.

The next experiment demonstrated that acceleration of clinical signs of EAE by adrenalectomy was accompanied by acceleration of appearance of inflammatory CNS lesions, and was not a mere unmasking of latent disease. Preliminary experiments revealed that the spleen could be used for passive transfer, and its considerable size facilitated preparation of large pools of cells. Three spleen cell pools were prepared from donors with EAE. Sixteen adrenalectomized and 16 intact recipient rats were injected alternately with equal portions of the pools. Donor/recipient varied from 1/2 to 1/5. By the third day after injection, all adrenalectomized but only 2 intact recipients had clinical signs of EAE (Table II). At this point all the rats were sacrificed. Histologic study revealed far more EAE lesions in adrenalectomized than in intact recipients (Table II). Although adrenalectomy had definitely accelerated the passive transfer of EAE, the presence of mild lesions in most intact controls, and the results of the first experiment, suggested that EAE would have progressed in the intact rats if longer survival had been permitted.

In contrast to these results, adrenalectomy of *donors* had no effect on passive transfer. In one trial, lymph node cells taken shortly after onset of EAE signs from 9 adrenalectomized and 8 intact donors were equally potent (donor/recipient = 1/1).

Discussion. Success of passive transfer may depend on survival with functional activity (5) and perhaps multiplication of donor lymphoid cells in the recipient. If this is so, the accelerating effect of adrenalectomy of the recipient may be due to removal of corticosteroid-induced lymphocytokaryorrhexis

TABLE II. Passive Transfer of EAE with Spleen Cells: Acceleration by Adrenalectomy.

Spleen cell pool	No. of cells to each recipient	Recipients Type	No.	EAE in recipients: Clinical, day of onset*	EAE in recipients: Histologic scores†
1	4.7×10^8	Adrenex	2	2 3	4 3
		Intact	2	3 –	4 1
2	1.6×10^8	Adrenex	5	2 2 3 3 3	4 4 3 3 2
		Intact	5	3 – – – –	2 1 1 0 0
3	1.6×10^8	Adrenex	9	2 2 2 3 3 3 3 3 3	3 3 3 3 2 2 2 2 1
		Intact	9	– – – – – – – – –	2 1 1 1 1 1 1 1 0

* Each figure represents a single rat with clinical signs that appeared on the indicated day; each hyphen represents a single rat without signs.

† Each figure is score of a single rat, graded from 0 to 4 according to number and intensity of lesions on randomized slides read without knowledge of the experimental group.

and mitotic inhibition(6).

Although our experiments have demonstrated only an accelerating influence of adrenalectomy, there may be situations in which the number or potency of lymphoid cells is so close to a threshold value, that passive transfer is successful *only* with the aid of adrenalectomy of recipients. This true enhancement has not yet been observed in our experiments.

Summary. Passive transfer of EAE has been accomplished with spleen as well as lymph node cells from actively sensitized donors. Adrenalectomy of recipients accelerated passive transfer of EAE. Adrenalectomy also accelerated active induction of EAE, especially when surgery was performed at least 2 days before injection of the antigen-adjuvant mixture.

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Neutralizing Antibodies to Simian Virus 40 (SV40) in Human Sera From India.* (30765)

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In some regions of north India, especially in the state of Uttar Pradesh (U.P.), man and the rhesus monkey live in close ecological association. In these areas, the contact of man with rhesus varies from maximal as with rhesus dwelling in crowded bazaars, to little as with rural roadside groups of rhesus. An educated guess of the number of rhesus in U.P. in 1960 was 800,000, and it was estimated that more than 80% of these lived in association with human communities(1): the human population of U.P. was 73.7 millions

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